Monocloning Oligoclonality – Dissecting the Humoral Immune Response in Central Nervous System Inflammations

Dissertation

zur

Erlangung der naturwissenschaftlichen Doktorwürde
(Dr.sc.nat.)

vorgelegt der

Mathematisch-naturwissenschaftlichen Fakultät

der

Universität Zürich

von

Sandra Künzle

aus

Zürich

Promotionskomitee

Prof. Dr. Peter Sonderegger
Prof. Dr. Adriano Fontana
Prof. Dr. Norbert Goebels
Prof. Dr. Burkhard Becher

Zürich, 2007

SUMMARY

The immune system is involved in the course of numerous diseases of the central nervous system (CNS). On the one hand, beneficial immune responses play an important role in the control of infectious agents. On the other hand, detrimental immune responses can be misdirected against structures of the body's self. Multiple Sclerosis (MS), for example, is an autoimmune disorder of the CNS involving immune responses directed against CNS self-antigens. A common characteristic of MS and infectious CNS diseases like neuroborreliosis (NB) is the presence of an antigen driven immune response in the cerebrospinal fluid (CSF). This is exemplified by the presence of clonally expanded B cells and plasma cells (cePC) and their presumed products, oligoclonal immunoglobulins (Ig). These can be visualized as oligoclonal bands (OCB) on isoelectric focussing gels. While it is known that in CNS infections at least some of the OCB are directed against the causative infectious agent, the specificity and disease relevance of OCB in chronic inflammatory autoimmune diseases of the CNS, such as MS, remain unknown.

The goal of this work was to dissect the antigen-specific, humoral immune response in the CSF down to a single cell level in order to investigate its potential role played in inflammatory disorders of the CNS such as NB or MS.

Individual, CD138+, FACS sorted CSF plasma cells were used as the basis for the present studies. Starting with single cell RT-PCR of Ig genes (heavy and light chains) of CSF plasma cells from a patient with NB we identified expanded plasma cell clones. The antigen specificity of their secreted antibodies was resurrected by recombinant expression of correctly paired Ig heavy and light chain genes as monoclonal antibodies (mAb). As expected, we found specificity for the causative infectious agent *Borrelia burgdorferi* (Bb) among the cePC derived mAb. Interestingly however, we also obtained one mAb specific for human CNS myelin, without detectable cross-reactivity with Bb antigens, from an independent cePC of the same patient.

These findings indicate that in NB mechanisms may be at play that induce distinct antigen driven immune responses specific for pathogen and self-antigens independent from "molecular mimicry". Based on these results we conclude that the applied experimental strategy faithfully reproduces antigen specificities as found in CNS inflammations and that the resulting mAbs can be used for the analysis of

potential antigenic targets. Because the immunopathological relevance of cePC in the CSF of MS patients remains unknown, we moved on to produce mAbs from these cePCs. Immunofluorescence experiments indicate CNS reactivity of these mAbs. However, their cognate antigens have not been conclusively identified up to now. Future work is aimed at unraveling their antigen specificity and is expected to further elucidate underlying disease mechanisms in MS.

ZUSAMMENFASSUNG

Das Immunsystem spielt bei verschiedenen Krankheiten des zentralen Nervensystems (ZNS) eine wichtige Rolle. Einerseits bekämpft es infektiöse Erreger, anderseits kann irrtümlicherweise auch eine schädigende Immunreaktion gegen körpereigene Strukturen entstehen. Die Multiple Sklerose (MS) ist eine Autoimmunerkrankung, bei der das ZNS angegriffen wird. Sowohl bei der MS als auch bei infektiösen Erkrankungen des ZNS, wie zum Beispiel der Neuroborreliose (NB), können im Liquor cerebrospinalis Spuren einer Antigen-induzierten Immunantwort nachgewiesen werden. Dazu gehören klonal expandierte B- und Plasmazellen sowie oligoklonale Immunoglobuline, die auf isoelektrisch fokussierten Gelen als oligoklonale Banden (OKB) dargestellt werden können. Wie bereits gezeigt werden konnte, sind bei chronischen ZNS Infektionen ein Teil der OKB gegen den zugrunde liegenden Erreger gerichtet. Jedoch ist die Spezifität und Krankheitsrelevanz von OKB bei chronischen Entzündungen des ZNS, wie bei der MS, nach wie vor unbekannt.

Das Ziel dieser Arbeit war, die bei der NB und MS im ZNS stattfindende Antigen-induzierte, humorale Immunantwort, bis auf die Ebene der von Plasmazellklonen produzierten Antikörpern zu "zerlegen". Hierfür wurde ein System zur Herstellung rekombinanter Antikörper etabliert, deren Antigenspezifität und Affinität exakt den Antikörpern entspricht, welche von klonal expandierten Plasmazellen im Liquor von Patienten produziert werden.

Einzelne, CD138+, FACS sortierte Plasmazellen aus dem Liquor waren die Basis der hier präsentierten Studien. RT-PCR der Immunoglobulingene (IgG, schwere und leichte Ketten) einzelner Plasmazellen ermöglichte die Identifizierung expandierter Plasmazellklone. Durch die rekombinante Produktion monoklonaler Antikörper (mAk) mit korrekt gepaarten schweren und leichten Immunoglobulinketten, wurde die ursprüngliche Antigenspezifität rekonstruiert.

Zunächst wurden verschiedene mAk klonal expandierter Liquor-Plasmazellen eines Patienten mit NB hergestellt. Wie erwartet, fand sich unter diesen ein Antikörper mit Spezifität für den zugrunde liegenden infektiösen Erreger *Borrelia burgdorferi* (Bb).

Interessanterweise zeigte jedoch ein mAk eines anderen Plasmazellklons Spezifität für ZNS Myelin, ohne dass dieser Antikörper mit Bb Antigenen kreuzreagierte.

Diese Ergebnisse weisen darauf hin, dass in NB Mechanismen eine Rolle spielen, welche unabhängig von “molecular mimicry“ eine Antigen-induzierte Immunantwort sowohl gegen den Erreger als auch gegen Autoantigene hervorrufen. Da diese Resultate zudem die Anwendbarkeit der hier präsentierten experimentellen Strategie eindeutig nachweisen, wurde das Augenmerk zusätzlich auf Plasmazellen von MS Patienten gerichtet. Dies insbesondere deshalb, da die Ziel-Antigene und immunopathologische Relevanz der im Liquor zu findenden Plasmazellen von MS Patienten bis anhin unbekannt sind. Erste Resultate zeigen, dass mAk, welche von Plasmazell-Klonen aus dem Liquor von MS Patienten rekonstruiert wurden, in Immunofluoreszenzfärbungen eine ZNS Reaktivität aufweisen. Zukünftige Arbeit beabsichtigt die Identifizierung der Antigene und könnte dadurch helfen, die der MS zugrunde liegenden Krankheitsmechanismen besser zu verstehen.

1 INTRODUCTION

1.1 The humoral immune response in central nervous system inflammations

1.1.1 Introduction

Inflammation is the first response of the immune system to danger signals evoked by infection or irritation. It is manifested by invasion of circulating immune cells into the inflamed tissue and induction or activation of inflammatory mediators.
For a long time, the central nervous system (CNS) was considered to be "immune privileged" in the sense of not contributing to inflammation. This dogma is no longer tenable. The CNS does exhibit features that suggest active involvement in inflammation, and in response to injury, infection or disease, resident CNS cells can generate inflammatory mediators and recruit immune cells.
Two major populations of lymphocytes - B and T cells - are involved in the adaptive and specific immune response. They are responsible for the humoral and cell-mediated branches of the immune system. The present studies focuses on the humoral immune response produced by B cells. The inflammatory response has a clear benefit in infectious states, when activated in a regular manner for a defined period of time. However, sustained, excessive or inappropriate inflammation can be a major cause for the damage of tissue in several diseases, including Multiple Sclerosis (MS) and neuroborreliosis (NB).

1.1.2 B cell development

B cells carry immunoglobulin (Ig) receptors on their surface, are professional antigen-presenting cells and may evolve to plasma cells upon receiving appropriate stimulatory signals. B cells emerge from hematopoietic stem cells in the bone marrow. In germ-line DNA, multiple gene segments encode portions of a single Ig heavy (H) or light (L) chain. In the case of H chains, variable (V), diversifying (D) and joining (J) gene segments are available for the generation of the antigen binding

region, while in the case of L chains only V and J regions are encoded in the germline. These gene segments cannot be transcribed and translated into H and L chains until they are rearranged into functional genes. During B cell maturation, Ig gene segments are randomly shuffled by a dynamic genetic system, capable of generating more than 10^{10} combinations (Fig. 1A). A mature immunocompetent B cell will contain a single functional H-chain variable (V)-region sequence and a single functional L-chain V-region sequence, so that the individual B cell is antigenically committed to a specific epitope. The joining of the coding sequences is often imprecise and random nucleotides can be added. As a result each B cell carries a unique Ig sequence, which is shared only with the successive B cells resulting from proliferation. After different selection mechanisms (clonal deletion, receptor editing) contributing to B-cell tolerance, surviving naive immature B cells migrate to secondary lymphatic organs, where they undergo further maturation processes. The mechanisms for eliminating auto-reactive B cells are very stringent; nevertheless, some B cells bearing antibodies specific for self-proteins escape the control of the immune system.

B cells that encounter their specific antigen become activated and proliferate. The expansion of an antigen-activated B cell leads to a clone of plasma cells and/or memory B cells (Fig. 1B). All cells in the expanded clone bear similar Ig gene sequences and are therefore specific for the original antigen. Hence clonal expansion of a B cell is an unambiguous result of an antigen driven immune response.

B cell proliferation is accompanied by further differentiation and takes place in specialized areas of lymphoid follicles, so called germinal centers. The modifications include somatic hypermutation (alteration of the V-regions of Ig genes), affinity maturation (selection for B cells with high affinity for the antigen) and isotype-switching (alteration of the effector function of the expressed antibodies by changing the isotype) (Shapiro-Shelef and Calame 2005). B cells in the CNS during inflammation typically show somatic hypermutations suggesting that they matured in a germinal center and are thus the result of an antigen driven response (Baranzini et al. 1999; Baranzini et al. 2002; Owens et al. 1998; Qin et al. 1998; Smith-Jensen et al. 2000).

After germinal center maturation, B cells either develop into memory B cells or plasma cells. Plasma cells secrete high levels of antibodies, do not proliferate and can be short or long lived. Their ability to survive many years is an important feature

of the humoral immune response. They produce antibodies independently of an antigen re-stimulation but their survival is particularly dependent upon the environment (Manz et al. 2005).

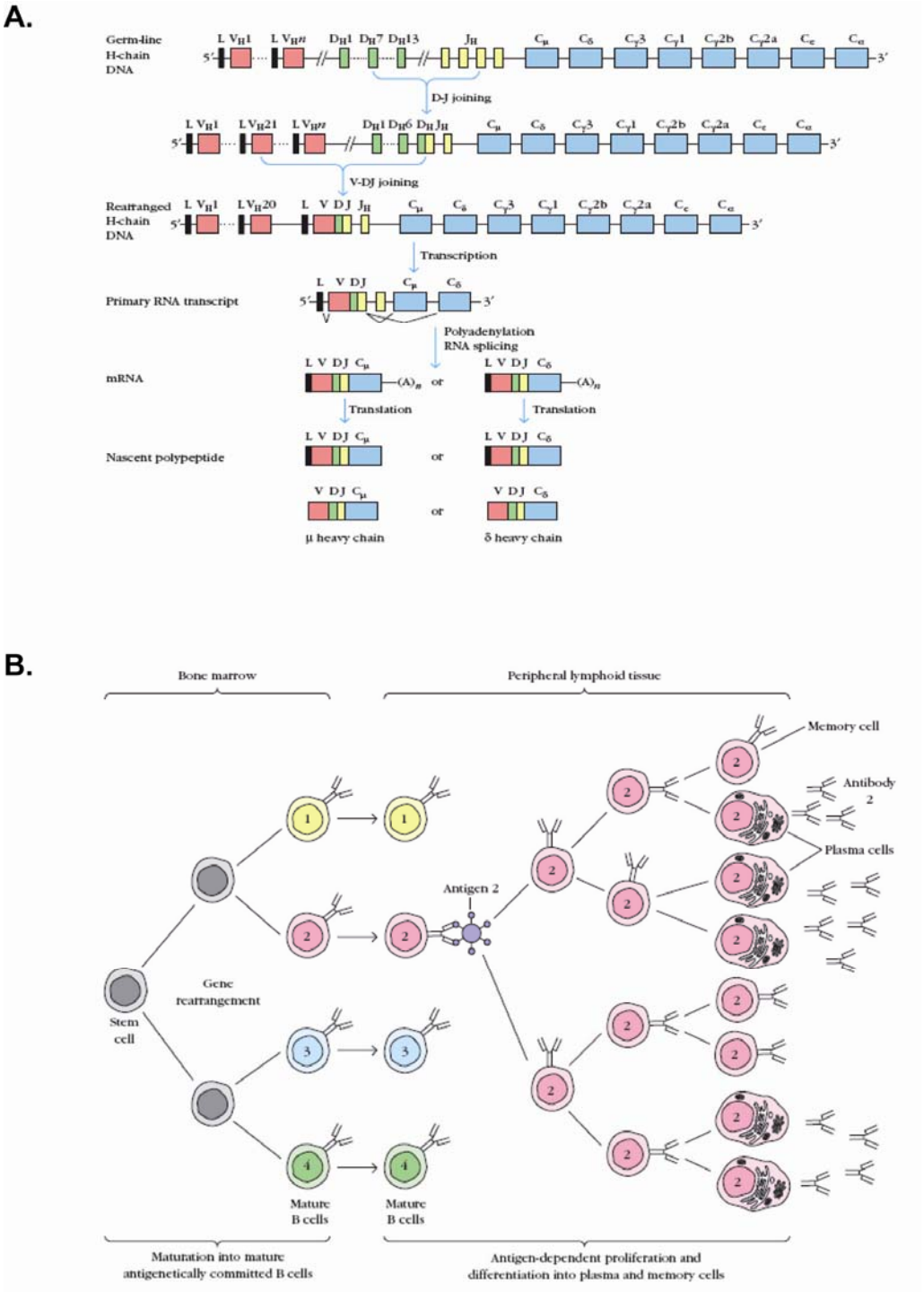

Figure 1: A. H-chain gene rearrangement and RNA processing events required to generate H chain proteins. Two DNA joining are necessary to generate a functional H-chain gene: a D_H to J_H joining and a V_H to D_HJ_H joining. B. Maturation and clonal selection of B cell lymphocytes. Clonal expansion of an antigen-activated B cell (number 2 in this example) leads to a clone of plasma cells and memory B cells. Adapted from Kuby Immunology (Richard A.Goldsby, Thomas J.Kindt, and Barbara A.Osborne 2000).

1.1.3 The CNS as a B cell supporting environment

There is more and more evidence that the brain is a place permissive for B cell and plasma cell survival and that in CNS inflammation the brain tissue can even act as a B cell supporting micro-environment.

The inflamed CNS provides a number of survival factors for B cells and plasma cells like BAFF and CXCL12. BAFF is one of the crucial factors for B cell survival (Schneider 2005). It is locally produced by astrocytes and up-regulated in active MS lesions (Krumbholz et al. 2005). CXCL12 plays a role as survival factor for plasma cells (Shapiro-Shelef and Calame 2005). It is constitutively generated by endothelial cells in the CNS and is also up-regulated in MS lesions (Krumbholz et al. 2006). Beside BAFF and CXCL12, many more cytokines promoting B cell differentiation and survival are abundant in MS lesions (Cannella and Raine 1995).

B cell migration to the CNS is still poorly understood but recent studies suggest that the homeostatic chemokines CXCL12 and CXCL13 play a role in B cell trafficking to the inflamed CNS (Krumbholz et al. 2006; Ransohoff, et al. 2003).

Clonal expansion of B cells has been detected in the cerebrospinal fluid (CSF) of patients with monosymptomatic optic neuritis (Haubold et al. 2004), MS (Baranzini et al. 1999; Colombo et al. 2000; Qin et al. 1998; Ritchie et al. 2004) and in patients with CNS infections like subacute sclerosis panencephalitis (SSPE) (Burgoon et al. 1999). However, the anatomical site where these B cell expansions occur, remains undetermined. Serafini et al. identified intrameningeal follicles in MS patients, thus providing evidence for germinal center reactions in inflamed CNS tissue itself (Aloisi and Pujol-Borrell 2006; Serafini et al. 2004). The concept of B cell differentiation in ectopic follicles in the CNS is further supported by the finding of B cell differentiation stages as they occur only in the germinal center of secondary lymphoid follicles, in the CSF of patients with MS and other acute inflammatory neurological disorders (Corcione et al. 2004). It is probable that organized B cell follicles with germinal centers in the inflamed CNS favor the B cell response sustaining clonal expansion of plasma cells and intrathecal antibody production.

1.1.4 Oligoclonal immunoglobulins in the cerebrospinal fluid

In the CSF of inflamed CNS, Ig can often be visualized as distinct oligoclonal bands (OCB) by isoelectric focusing of CSF proteins. The presence of OCB in the CSF but absence in matched serum samples is characteristic for an intrathecal synthesis (Fig. 2). OCB in CSF samples suggest that the B cell population in this compartment is not heterogenous but clonally restricted. It is most likely that clonally expanded plasma cells (cePCs) in the CSF are the source for the antibodies of OCB.

OCB can often be detected in the CSF of patients with autoimmune or infectious inflammatory CNS diseases. In infectious CNS diseases, the OCB are typically directed against the causative agent (reviewed in (Gilden et al. 1996)). In the case of NB it has been shown that the OCB in the CSF contain antibodies directed against *Borrelia burgdorferi* (Bb) (Hansen et al. 1990; Martin et al. 1988a).

In contrast to infectious CNS disease, the antigen specificity and pathogenic role of OCB in presumed autoimmune conditions such as MS remains to be defined. The intrathecal Ig production with the formation of OCB is a key feature of MS and the OCB remain stable for many years (Walsh and Tourtellotte 1986). The finding of a persistent OCB pattern supports the hypothesis that the CNS can provide a long-term survival niche for plasma cells. Although different factors in the CSF of MS patients (elevated Ig levels, OCB, anti-myelin antibodies) suggest that antibodies play an important role in MS, their relevance and impact on MS pathogenesis still remain elusive (Martin and Monson 2007). The antigen specificity of the OCB in MS is one of the most outstanding questions concerning their pathological relevance and could possibly clarify underlying disease mechanisms of MS.

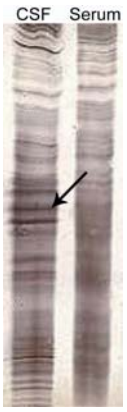

Figure 2: Isoelectric focussing gel of CSF and serum proteins (silver stain). Arrow points to one of the OCB present in the CSF but not in the serum of the same patient.

1.2 Neuroborreliosis

1.2.1 Introduction

Lyme borreliosis or Lyme disease is a term to describe the various disease states of infection with tick- or insect-borne borrelia bacteria. The pattern of clinical manifestations of Lyme borreliosis spread over four organ systems, the skin, the nervous system, the joints, and the heart.

1.2.2 History

Lyme borreliosis was first described in 1975 in the town of Lyme (Connecticut, USA) and has now become a widespread infectious disease in the USA and Eurasia. While a connection of the disease with ticks has long been assumed, only in 1982 was a novel spirochete first isolated from ticks and subsequently from patients with Lyme disease (Burgdorfer et al. 1982; Benach et al. 1983). The spirochete was named Borrelia burgdorferi in honor of its discoverer Willy Burgdorfer, an expert in tick-borne diseases at Rocky Mountain Labs in Hamilton, Montana.

1.2.3 Epidemiology

Lyme borreliosis occurs mainly in the northern hemisphere from North America to Europe and throughout Asia. The underlying infectious agent Bb is usually transmitted by the bite of certain ticks of the species Ixodes. The tick has a two year three-stage life cycle (larval, nymphal, adult) and feeds once during each stage (Lane et al. 1991). The tick may become infected at any stage of its life cycle by feeding on a host that is a natural reservoir for Bb. The main hosts of borrelia are mice, dormice, deer, voles as well as lizards (Hengge et al. 2003). In Europe, the chance of becoming ill due to Bb after a tick bite is probably below 1% (Paul, et al. 1987). Several factors play a role in the transmission of Bb from ticks to human beings. On the one hand, the proportion of infected ticks in a certain geographic area is relevant. On the other hand, the risk of transmission of Bb from an infected Ixodes tick is related to the duration of feeding.

1.2.4 Causative agent: *Borrelia burgdorferi*

The spirochaetal bacteria causing Lyme borreliosis belong to the genus Borrelia and further to the species of *Borrelia burgdorferi* sensu lato. The most prominent disease causing genospecies are *Borrelia burgdorferi sensu stricto*, *Borrelia afzelii* and *Borrelia garinii*. The ultrastructure of Borrelia species resembles other spirochetes characterized by an axial filament composed of flagella, which runs lengthways between the cell wall and outer membrane. This allows the spirochete to move in a corkscrew fashion (Barbour and Hayes 1986). Bb is a Gram-negative organism that is 10-30 um long. It can be cultured in liquid cultures in Barbour-Stoenner-Kelly (BSK) II medium and has under optimal conditions (30-34°C, microaerophilic milieu) a generation time of 7 to 20 hours. Important antigens of Bb are the 41 kD flagellin protein, several outer surface proteins (Osp A-F) and a 60 kD antigen, which has been termed "common antigen" and belongs to the heat shock protein family. The genome of Bb (strain B31) has been sequenced (Fraser et al. 1997). It includes a linear chromosome as well as 9 linear and 12 circular plasmids - by far the largest number of plasmids found in any known bacterium (Casjens et al. 2000).

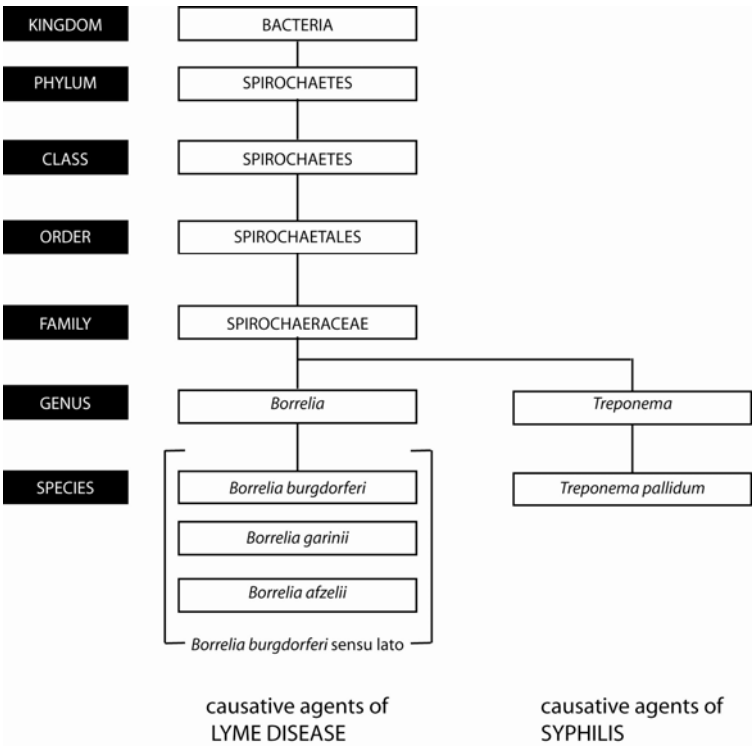

Figure 3: Borrelia Taxonomy

1.2.5 Clinical manifestation

Lyme borreliosis has many signs and symptoms and the clinical manifestations spread over four organ systems, the skin, the heart, the joints and the nervous system. The incubation period from infection to the onset of symptoms is usually 1-2 weeks, but can be much shorter or even as long as one month. The course of the disease can be divided into an acute or early (the first weeks or month) and a chronic or late phase (after several month or years) (Pfister et al. 1994).

Skin: The most common manifestation of early Lyme borreliosis is erythema migrans, which is a rash that spreads out centrifugally, generally at the site of a tick bite 3 to 30 days after the bite. It is sometimes homogeneous but usually it is ring-shaped (Louis Reik 1991).

Heart: Cardiac complications of Lyme borreliosis may occur in up to 8% of patients. The most common feature of Lyme carditis is a transient atrioventricular block of various degrees (Nagi et al. 1996).

Joints: About 60% of untreated patients with erythema migrans in the USA develop arthritis (Steere et al. 1987). The brief attacks of arthritis occur weeks to months after the initial infection and some patient develop persistent joint inflammation.

Nervous system: Neurological features of lyme borreliosis or NB include the Bannwarth syndrome (meningoradiculoneuritis), meningitis and cranial nerve palsies. The Bannwarth syndrome represents the second most common clinical manifestation of acute borrelia infection in Europe after erythema migrans and is characterized by CSF lymphocyte pleocytosis and intense radicular pain (Kaiser 1998). Frequently, these patients have also sensitive irritations and an occasional paresis is characteristically asymmetrically. In three-quarters of patients with Bannwarth syndrome further neurological symptoms occur after 1-4 weeks.

Cranial nerve palsies have been described for different cranial nerves but by far the most common is an involvement of the facial nerve. Unilateral and bilateral facial paralysis may occur in up to 11% of patients with Lyme disease (Clark et al. 1985).

Occasionally, a chronic encephalomyelitis develops with marked intrathecal production of antibodies against the spirochete (Oschmann et al. 1998). Magnetic-resonance imaging usually shows diffuse white matter lesions (Fig. 4). Main differences to MS are the presence of borrelia specific antibodies in the CSF and usually a higher cell count and more pronounced impairment of the blood-CSF

barrier. It is still unknown, if the chronic neurological involvements in NB are due to persistent Bb infection or the result of an aggressive autoimmune response. Further research is necessary to clarify the underlying mechanisms.

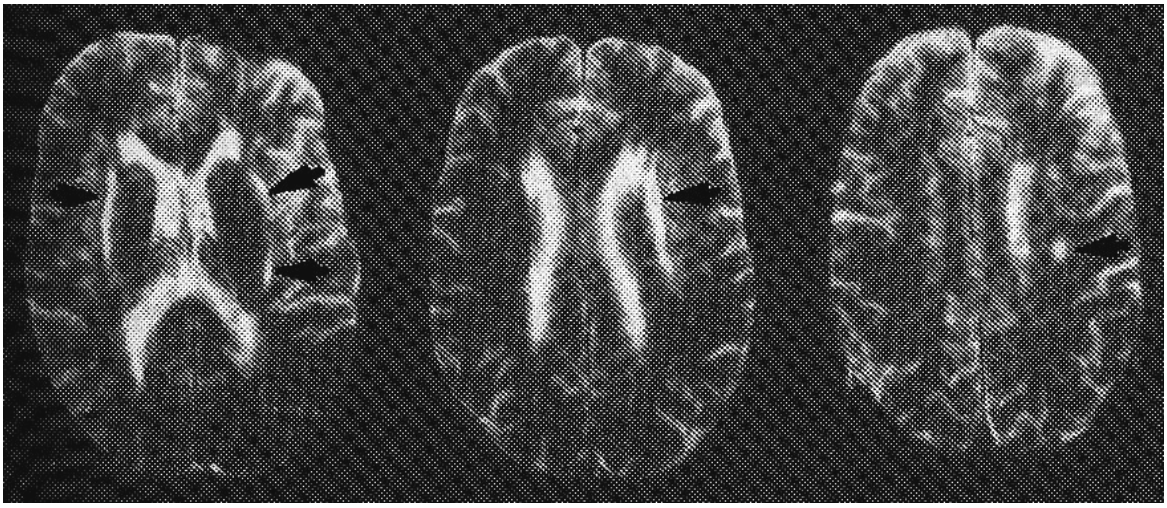

Figure 4: Magnetic resonance imaging scans of the brain of a 49-year-old man with chronic progressive Lyme encephalomyelitis. The patient had tetraspastic syndrome and ataxia of 1 1/2 years' duration. CSF showed lymphocyte pleocytosis and intrathecal Bb antibody production. Arrows indicate periventricular lesions of the white matter. Adapted from Pfister *et al.* 1994 (Pfister et al.1994)

1.2.6 Diagnosis

The diagnosis of Lyme borreliosis is based on clinical symptoms and laboratory data. In locations where Lyme borreliosis is endemic, the clinical diagnosis of erythema migrans, which does not occur in all cases, is sufficient. Culturing Bb from specimens (from erythema migrans, plasma samples, CSF) allows a definitive diagnosis but is very difficult. Likewise a PCR for detecting Bb DNA can be made. But most important are serological test measuring the body's immune system response, including enzyme-linked immunosorbent assay (ELISA), indirect immunofluorescence assay and western blot.

For diagnosing NB, both serum and CSF have to be tested and the diagnosis is supported by the demonstration of an intrathecal synthesis of borrelia-specific antibodies in the CSF. Depending on the constellation of clinical and laboratory data, the diagnosis of NB is possible, likely or certain (Kaiser 1998).

1.2.7 Treatment

Traditionally lyme borreliosis is treated with a course of antibiotics. Most treated patients have an excellent prognosis, though some patients with NB may suffer from residual deficits and/or progression of the disease (Logigian et al. 1990).

1.2.8 Immune response and autoimmunity

The innate and the acquired immune response play both an important role in the recognition and elimination of foreign microorganisms. While innate immunity depends on a number of barriers that are effective against a wide variety of pathogens, adaptive immunity is specific for particular microorganisms or foreign molecules.

A key component of the innate immune response is the complement system. It is composed of a collection of serum proteins and cell surface receptors that are part of the initial immune response to infection by bacteria, fungi, viruses or parasites. There are three pathways of complement activation. Two of them - the alternative and the mannan-binding lectin pathway - are initiated by the interaction of complement components with the surface of microorganisms. The third pathway - the classical activation pathway - requires the formation of antigen-antibody complexes on the surface of the microorganism. The three main consequences of complement activation are opsonization of pathogens, the recruitment of inflammatory cells and direct killing of pathogens.

Bb can activate the classical and alternative pathway of the complement cascade in normal human serum, but is resistant to complement-mediated lysis (Kochi et al. 1991). This resistance results from the binding of the complement inhibitor factor H to proteins expressed on the surface of Bb and the subsequent inactivation of complement component C3b (Alitalo et al. 2001; Alitalo et al. 2002; Kraiczy et al. 2003).

Bb can be ingested and killed by different human phagocytic cells. The phagocytosis of the spirochetes can be both independent of opsonization with antibody and mediated by the Fc receptor (Montgomery et al. 1994; Peterson et al. 1984).

The acquired immune response encompasses the generation of clonally selected effector and memory T and B cells.

In patients with NB, activated T cells are recruited into the CSF compartment during acute infection of the nervous system (Jacobsen et al. 2003; Rupprecht et al. 2005a; Rupprecht et al. 2005b). Studies with CSF derived T cells demonstrated clones reactive for Bb derived antigens but also other clones specific for CNS self-antigens (Martin et al. 1988b). Further studies found Bb specific T cells cross-reacting with self-antigens suggesting molecular mimicry to be responsible for autoimmune mechanisms leading to chronic disease manifestation (Lunemann et al. 2006; Hemmer et al. 1999).

In NB, the immune response is highly focused to the CNS, involving pleocytosis, disruption of the blood-brain barrier and intrathecal antibody synthesis (Pfister et al. 1994; Wilske et al. 1986).

Various lymphomononuclear cells accumulate in the CSF of patients with NB, most typically plasma cells (Cepok et al. 2003; Sindern and Malin 1995).

Furthermore, high levels of IL-6, IL-8 and IL-10 are found in the CSF (Cepok et al. 2003). These interleukines promote the development of a humoral immune response: IL-10 stimulates B-cell proliferation and differentiation and induces the secretion of Igs (Moore et al. 2001). IL-6 induces the final maturation of B cells into Ig secreting plasma cells, favoring the secretion of the IgG1 subtype in particular (Taga and Kishimoto 1992). IL-8 is a chemotactic factor for various immune cells (Kimata et al. 1992). The combined effect of these cytokines may lead to pleocytosis and supports differentiation of B cells.

In NB, the levels of distinct Ig clones in the CSF are elevated and can be demonstrated as OCB by isoelectric focussing (Kruger et al. 1981). The finding of OCB and an elevated Ig index in the CSF point to a local production of the Ig in the CSF compartment. Analysis of Ig in the CSF has revealed specificity for Bb (Hansen, et al. 1990; Martin et al. 1988a) but also for CNS auto-antigens (Kaiser 1995).

Bb specific antibodies can persist for 10-20 years (Kalish et al. 2001) and Ig in sera of patients with lyme disease and of mice infected with Bb, have been demonstrated to protect against infection with Bb by passive immunization (Barthold and Bockenstedt 1993; Fikrig et al. 1994).

Different neural auto-antibodies that cross-react with Bb have been described. Sigal and colleagues identified an antibody specific for Bb flagellin that cross-reacts with

the heat shock protein 60 and binds to human peripheral nerve (Dai et al. 1993). The same antibody was also found to bind neuroblastoma cell lines and to slow down neurite outgrowth in culture (Sigal and Williams 1997). Another study described antibodies specific for Bb OspA proteins that cross-react with neurons in human neural tissue (Alaedini and Latov 2005). These studies support the hypothesis that autoimmunity due to molecular mimicry may play a role in NB. Such mechanisms are also known to be important in Lyme arthritis where human LFA-1 can serve as a cross-reactive auto-antigen for Bb OspA-reactive Th1 cells, resulting in treatment resistant Lyme arthritis (Gross et al. 1998).
However, the underlying mechanisms leading to chronic NB are unknown. The role of autoimmunity either triggered by molecular mimicry or by other mechanisms has to be further studied.

1.2.9 Vaccines

Lyme disease vaccine development has targeted the OspA protein of Bb. Two commercial recombinant OspA vaccines were formulated and have been shown to be effective (Connolly and Benach 2005). However, these vaccines have now been withdrawn from the commercial market due to limited confidence in these products as a result of indications for cross-reactivity of OspA with self-antigens (Gross et al. 1998).

1.3 Multiple Sclerosis

1.3.1 Introduction

MS is one of the most common neurological diseases of young adults in Europe and North America (Noseworthy et al. 2000). MS patients characteristically display chronic multifocal sclerotic plaques in the CNS from which the disease gets its name. MS is thought to be caused by the interaction of multiple genetic and environmental factors, yet the initial causative trigger is still unknown.

1.3.2 Epidemiology

The prevalence of MS varies considerably around the world. The prevalence rate is highest in northern Europe, southern Australia and the middle part of North America (Noseworthy et al. 2000). The reasons for the variation in the prevalence of MS worldwide are not understood. However it is hypothesized that both environmental factors and population-specific genetics probably play a role.
Twin studies demonstrated increased concordance rates among monocygotic (25-30%) compared to dizygotic (2-5%) twins with MS (Kenealy et al. 2003). These data provide clear evidence for a genetic component in the disease susceptibility; however the concordance rate in monozygotic twins of significantly less than 100% also highlights the important contribution of environmental factors.

1.3.3 Clinical manifestation

MS typically begins in early adulthood and has a variable prognosis. The clinical manifestations are diverse, since every part of the CNS can be affected. Common presenting features include weakness, paraesthesia or focal sensory loss, optic neuritis, diplopia, ataxia and vertigo. Other manifestations can include painful muscle spasms, trigeminal neuralgia, fatigue and depression, subtle cognitive difficulties, psychiatric disturbances and seizures (Love 2006). Characteristic for MS are CNS white matter lesions and OCB of Ig detected by magnetic resonance imaging and elecrophoresis of cerebrospinal fluid, respectively. The MS disease course can be

either relapsing and remitting or it can be progressive. The latent phase between the first manifestation of MS and the first relapse can be many years and the intervals between relapses are variable. For the clinical diagnosis the revised criteria of McDonald et al. are most widely used (Polman et al. 2005).

1.3.4 Pathogenesis

The pathological hallmarks of MS are demyelinated plaques within the white matter combined with inflammatory infiltrates consisting of lymphocytes and activated macrophages. The demyelination is associated with oligodendrocyte loss. Gliosis with astrocyte proliferation and glial fibre production are also important pathological features of MS.

There is increasing evidence that neuroaxonal damage is a key feature in MS lesions and that it has a major impact on permanent neurologic deficits (Trapp et al. 1999) . It may occur either in parallel with myelin destruction or during a second phase, when the axon is demyelinated and more susceptible to damage.

There is a variable degree of remyelination in the plaques (Noseworthy et al. 2000) and oligodendrocyte precursor cells can be found (Wolswijk 1998). However, the mechanisms that promote endogenous remyelination are still poorly understood.

Pathological similarities between MS and the animal model of experimental allergic encephalomyelitis and the detection of auto-reactive T and B cells in MS patients provide evidence that MS could be an autoimmune disease.

Activated auto-reactive T cells are thought to enter the blood brain barrier, and different factors like up-regulated adhesions molecules on the endothelial cells or activated proteases can facilitate their entry into the CNS. The T cells can then get stimulated further or become anergic by the recognition of their target antigen on antigen-presenting cells. Moreover they can stimulate B cells to produce auto-aggressive (or repair enhancing) antibodies. A cascade of events, which includes the production of different cytokines, can then result in the immune mediated injury to myelin and oligodendrocytes (Fig. 5).

One possible mechanism leading to tissue injury includes the assistance of myelin specific antibodies. It has been shown that antibodies against myelin oligodendrocyte glycoprotein (MOG) can be found within MS lesions (Genain et al. 1999) and that MOG-specific antibodies can cause demyelination (Linington et al. 1988; Piddlesden

et al. 1993). A possible scenario is that these antibodies and their underlying B cells gain access to the CNS through the disrupted blood brain barrier after initial T cell mediated inflammatory responses.

However, the role of myelin specific antibodies is still debated as such antibodies have been detected in the serum and CSF of MS patients, but also in control subjects (Karni et al. 1999; Lindert et al. 1999; Mantegazza et al. 2004; Markovic et al. 2003; Reindl et al. 1999; Sun et al. 1991; Xiao et al. 1991).

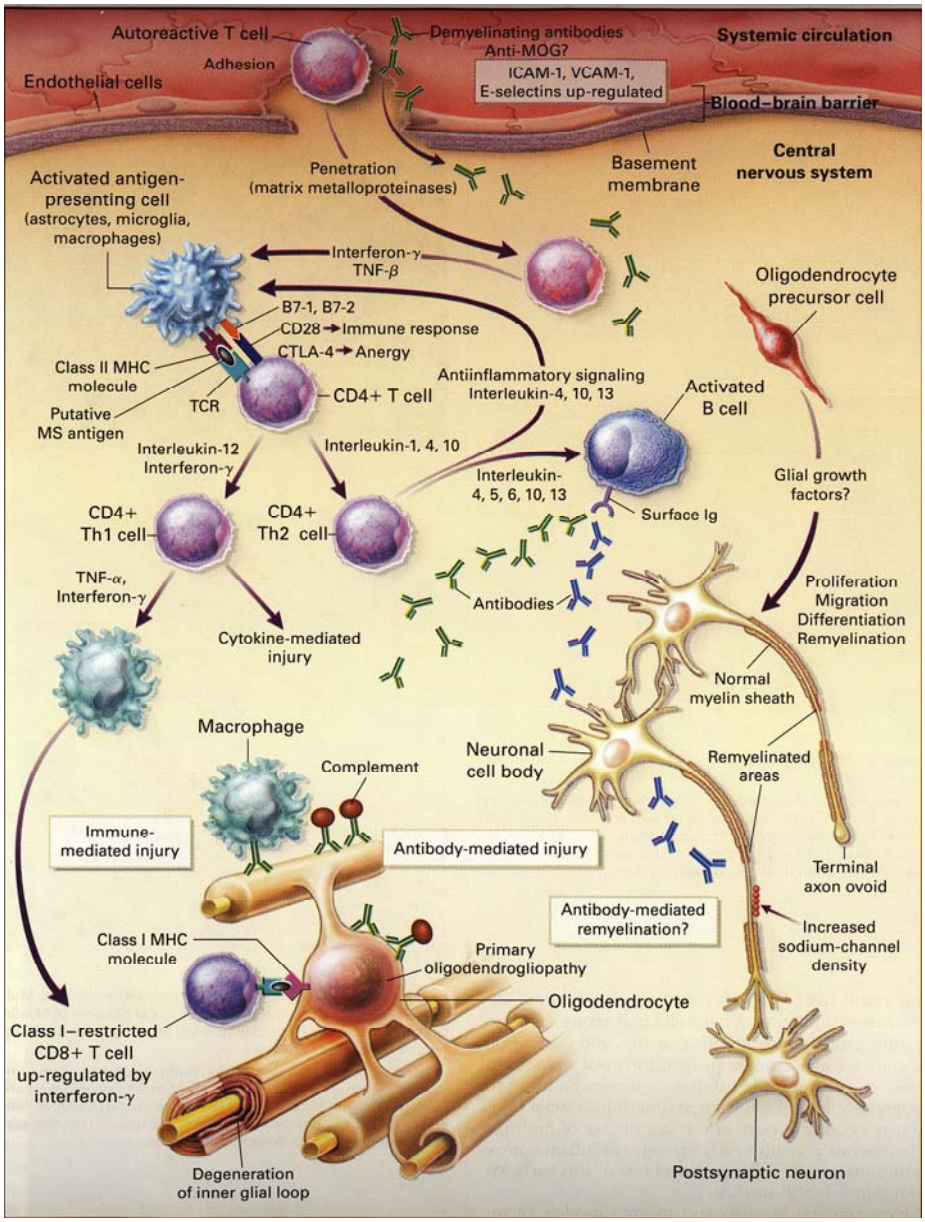

Figure 5: Possible Mechanisms of Injury and Repair in Multiple Sclerosis.

Genetic and environmental factors may contribute to the survival, activation and invasion of auto-reactive T cells and demyelinating antibodies into the central nervous system. Local factors and antigen dependent mechanisms play important roles in the immune responses leading to the injury and possible repair of myelin membranes. Adapted Noseworthy *et al*. (Noseworthy et al. 2000).

1.4 Goal of this work

A common characteristic of chronic inflammatory diseases of the CNS is the presence of oligoclonal Ig in the CSF, a feature suggesting the presence of a specific humoral antigen driven immune response in the CNS compartment. While it is known that in chronic CNS infections some of the OCB are directed against the causative infectious agent, the specificity and disease relevance of OCB in other chronic inflammatory diseases of the CNS such as MS remain unknown. So far the analysis of the antigen specificity and disease relevance of antibody responses in the CSF has been hampered by both the limited availability and oligo/polyclonal composition of CSF Ig.

The aim of this study was to dissect intrathecal antibody responses down to the single plasma cell level. By accurate sequence analysis we could identify plasma cells, which were - as a result of an antigen driven immune response - clonally expanded. Subsequent recombinant reconstruction of the antibodies originally produced by these cePC permitted their proper investigation.

Investigations with NB CSF were aimed on the one hand at evaluating our experimental set-up and on the other hand at investigating characteristics of the complex humoral immune response in NB. The production of antibodies from cePC of MS patients CSF was aimed at unraveling their antigen or tissue specificity.

Such knowledge could provide fundamental insights into the immunopathological role of antigen driven humoral immune responses in CNS inflammation.

2 RESULTS

2.1 Strategy for the production of recombinant antibodies from CSF derived clonally expanded plasma cells

2.1.1 Single cell RT-PCR

Our goal was to establish a system that allows the recombinant resurrection of antibodies originally produced by cePC. As a correct pairing of H and L chain Ig products is crucial for an antibody‘s specificity we had to obtain sequence information for both chains from each individual cell. The only way to achieve that is to amplify matching H and L chain Ig genes from the mRNA of a single cell. Figure 6 shows a schematic diagram of the method to produce recombinant antibodies originally secreted by single plasma cells. There are different families of Ig V region genes that can be used by a B cell, therefore we used for the amplification a mix of primers containing one for each of the different families. As the amount of mRNA of a single cell is very small, a highly sensitive system had to be established.

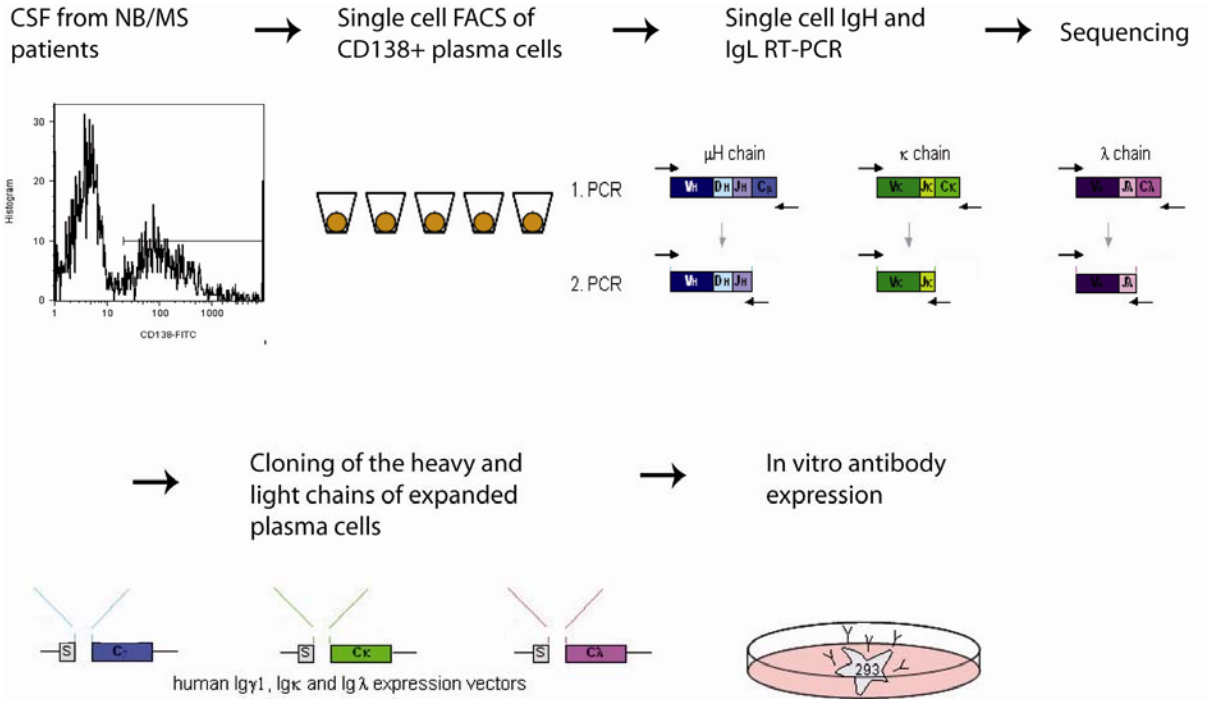

Figure 6: Schematic diagram of the method applied to produce recombinant antibodies with the same antigen specificity and affinity as the antibodies originally secreted by single plasma cells.

To establish single cell RT-PCR for the Ig genes from individual FACS sorted plasma cells, different protocols were initially tested. Different conditions and ingredients for the RT-PCR reaction as well as different primer sets were tested.

Single plasma cells were sorted directly into RT-buffer, immediately frozen on dry ice and stored at -80 °C until further use. For the amplification of the Ig V region genes a reaction mix composed of RT-buffer, dNTP, enzyme-mix, RNAse out and dH_20 was added to the plasma cell on ice. The cell was lysed by gently pipetting up and down and the mix was then split into three reactions containing gene-specific primers for either the H, the κ or the λ Ig chain. After reverse transcription reaction a first PCR amplification round was performed using PCR primers, specific for the Ig H, κ and λ Ig chain genes. The reverse primers, specific for the C regions of H, κ and λ Ig genes were used as described (Owens et al. 2003). As forward primers, a mix of primers specific for the conserved framework regions 1 of the different V region families of the Ig genes was used, which was adapted from Andris-Widhopf et al. (Andris-Widhopf et al. 2001).

Ig sequences were analyzed and assembled into groups of identical Ig H chain V region genes using SeqMan software (DNASTAR). If a variable region with a specific CDR3 region was amplified from more than one plasma cell, it was defined to be the product of a cePC. As a number of precautions had been taken to avoid cross-contamination between wells of PCR plates (see Materials and Methods), and we also obtained several "unique" sequences from single plasma cells analyzed in the same PCR experiment, we expected that sequences with 100% homology were not the product of contamination.

To obtain further information about potential maturations of the antibodies from cePC we analyzed their sequences for germline segment usage and their germline homology via the V-Quest tool on the IMGT website (Giudicelli et al. 2004). As further described in the results section, we found significant variations in the expressed segments compared to the germline segments indicating that processes of affinity maturation had occurred.

2.1.2 Cloning and recombinant expression of immunoglobulin amplicons

For the recombinant expression of the cePC derived Ig genes, we used the system described by Wardemann *et al.* (Wardemann et al. 2003), which allows the generation of recombinant human monoclonal antibodies (mAb) with a human IgG1 backbone using a eukaryotic expression system.

Therefore the PCR H and L chain Ig amplicons from cePCs were reamplified in a nested second round PCR with primers containing restriction sites (Wardemann et al. 2003). After digesting, these products were cloned into vectors containing the Ig H, κ or λ C regions (Vectors were kindly provided by H. Wardemann, Max-Planck Institut für Infektionsbiologie, Berlin, Germany).

To produce the recombinant antibodies we transiently transfected 293T human embryonic kidney cells with both plasmids coding for the H and L chain Ig of the desired antibody. Eight days after transfection and culture in serum free medium, we purified the antibodies from the supernatant using proteinG column on Fast Liquid Protein Chromatography. Figure 7 shows a SDS-gel of purified antibodies stained with silver to demonstrate their purity.

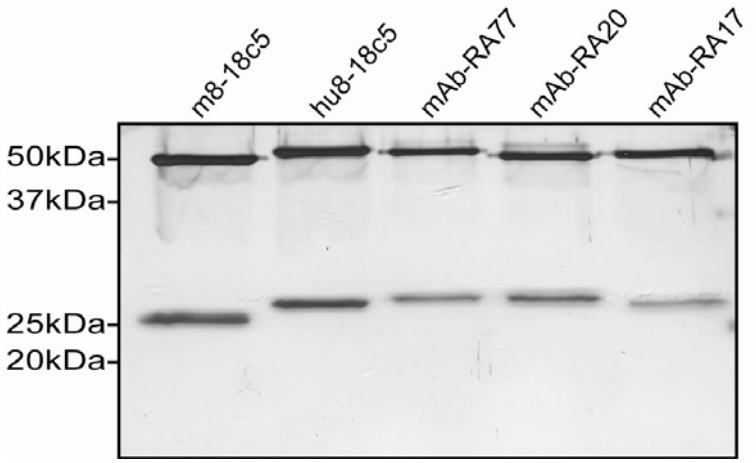

Figure 7: High purity of generated human recombinant monoclonal antibodies. 12% acrylamide gel run under reducing conditions and stained with silver. 100ng of antibodies were loaded after FPLC purification.

2.1.3 Proof of principle: recombinant expression maintains specificity and affinity

In pilot experiments we evaluated the fidelity of the recombinant expression system described. We therefore amplified the V regions of the Ig genes of the well known MOG specific hybridoma antibody 8-18c5 and cloned them into the previously described expression vectors. We thereby produced a chimeric antibody (hu8-18c5) of the variable region of the originally murine 8-18c5 (m8-18c5) with a human IgG1 backbone. Using MOG coated ELISA we compared the avidity of both m8-18c5 and hu8-18c5 and could show that it is not changed by the process of recombinant expression (Fig. 8). These results confirm that we can faithfully conserve the specificity and affinity of the originally produced antibodies when producing them in the described recombinant system.

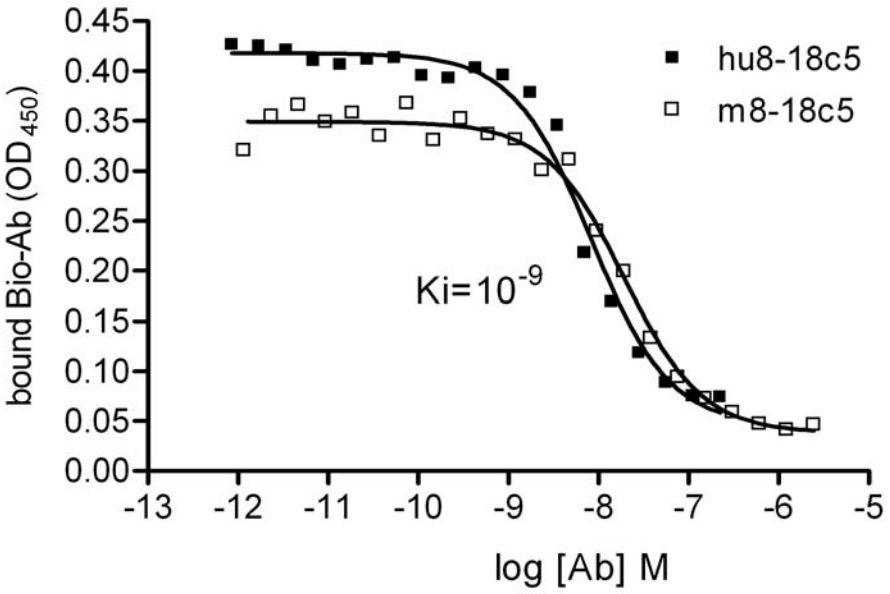

Figure 8: The avidity of m8-18c5 is maintained after cloning and recombinant expression as human chimeric hu8-18c5. Shown are the results of competition ELISAs of biotinylated vs. non-biotinylated m8-18c5 (□, Ki=10^{-9}) and biotinylated vs. non-biotinylated hu8-18c5 (■, Ki=10^{-9}) for their binding to recombinant MOG.

2.2 Production and characterization of recombinant antibodies from clonally expanded CSF plasma cells from a neuroborreliosis patient

As described in the introduction, a common characteristic of NB CSF is the presence of oligoclonal Ig, a feature suggesting the existence of a CSF specific antigen driven immune response. So far, the analysis of Ig in the CSF has revealed specificity for Bb (Hansen et al. 1990; Martin et al. 1988a) but also for CNS auto-antigens (Kaiser 1995). However, mechanisms underlying Bb directed humoral immune defense and CNS tissue damage still remain poorly understood and a more in-depth characterization of the focused antibody response in the CSF has been hampered by both the limited availability and oligo/polyclonal composition of CSF Ig.

To gain further information on the immunological properties of antigen driven immune responses in the CSF of patients with NB, we recombinantly reconstructed antibodies derived from cePCs of NB CSF.

Furthermore, as it is known that in NB part of the OCB are specific for Bb, we expected that some of the recombinant antibodies derived from cePcs - the putative source of the OCB antibodies - would be specific for Bb. We thereby wanted to verify that we are indeed able to produce the disease relevant antibodies with the applied method. With respect to the unraveling of the specificity of plasma cells from MS CSF with this method, this proof of principle is of major importance.

2.2.1 RT-PCR analysis of individual FACS sorted plasma cells from NB CSF demonstrates clonal expansion

Eighty-eight CD138+ plasma cells were sorted from the CSF of a patient with NB into wells of a 96 well PCR-plate at a ratio of 1 cell/well. Single cell RT-PCR of Ig genes yielded Ig H chain amplicons and corresponding sequence information from 54 out of the 88 (61.4%) individual plasma cells. By assembling the Ig H chain V region genes in groups of sequences sharing identical heavy chain CDR3 regions, we were able to discern 3 independent cePC (RA17, RA20 and RA77; Tab. 1). Matching Ig L chain gene amplicons were obtained for each of the 3 cePC.

In the V region sequences of RA17, RA20 and RA77 we found several mutations of the germline segments, which is a sign that affinity maturation processes took place for these antibodies (Tab.1).

Table 1 summarizes the frequencies of the expanded plasma cells and the characteristics of the corresponding V-H and V-L sequences.

			Closest Germline				
Clone	Frequency		V segment	% Homology	J segment	D segment	CDR3
RA77	4/54	H chain:	IGHV5-a	97.6	IGHJ5	IGHD2-15	AGEAAGTRIDRWFDP
		L chain:	IGLV9-49		IGLJ3		GADHGSGSNFVWV
RA20	3/54	H chain:	IGHV4-59	98.2	IGHJ6	IGHD5-24	ARVPGERDGYHYMAYAMDV
		L chain:	IGLV3-1		IGLJ3		QAWDSSTLGV
RA17	4/54	H chain:	IGHV5-a	97.8	IGHJ32	IGHD6-6	ASLAARGAFDI
		L chain:	IGLV3-25		IGLJ3		QSADSSGSYRV

Table 1: Characteristics of VH and VL region sequences of recombinant antibodies derived from expanded CD138+ CSF plasma cells from NB patient RA. Frequency indicates the number of the respective H chain sequences containing the indicated H chain CDR3 region among all plasma cells analyzed by PCR. Shown are the most homologous V, D and J germline segments, degree of homology to the closest germline segment for the H chain V region and the respective CDR3 amino acid sequence.

2.2.2 Recombinant antibody RA77 is specific for Borrelia flagellum protein p41

Correctly paired Ig H and L chain amplicons of each of the three cePC were cloned into the previously described eukaryotic expression vectors and expressed as recombinant human mAbs (RA77, RA20, RA17). In ELISA experiments RA77 showed clear reactivity with extracts of 4 different strains of Borrelia (*B. burgdorferi sensu strictu, B. afzelii, B. garinii, B. valaisiana*) while no binding to *E.coli* could be detected. No significant binding to any Borrelia strain could be detected for RA17 and RA20 (Fig. 9A). To further investigate Borrelia specificity of RA77 and rule out possible cross-reactivity with other spirochetes we performed immunofluorescence experiments with Bb and Tp attached to microscopy slides. While specific binding of RA77 to Bb could be confirmed, no binding to Tp could be detected (Fig. 9B-G).

In immunoprecipitation experiments using RA77 together with Bb lysate, a protein band of approximately 40 kDa was detectable in the precipitate. In the control immunoprecipitation with hIgG1 no bands in addition to the expected Ig H and L chain bands were detected (Fig. 10A). MALDI-TOF analysis of the 40 kDa band revealed peptide masses matching theoretically derived masses from tryptic-digested flagellin protein p41 of Bb. ELISA experiments with purified recombinant p41 flagellum protein confirmed the result from mass spectrometry (Fig. 10B). Competition experiments using RA77 and native Bb lysate demonstrated a high affinity interaction (K_i=1x10^{-9}). Interestingly, the interaction between RA77 and recombinant p41 yielded a 1000-fold lower affinity (K_i=1x10^{-6}) (Fig. 10C).

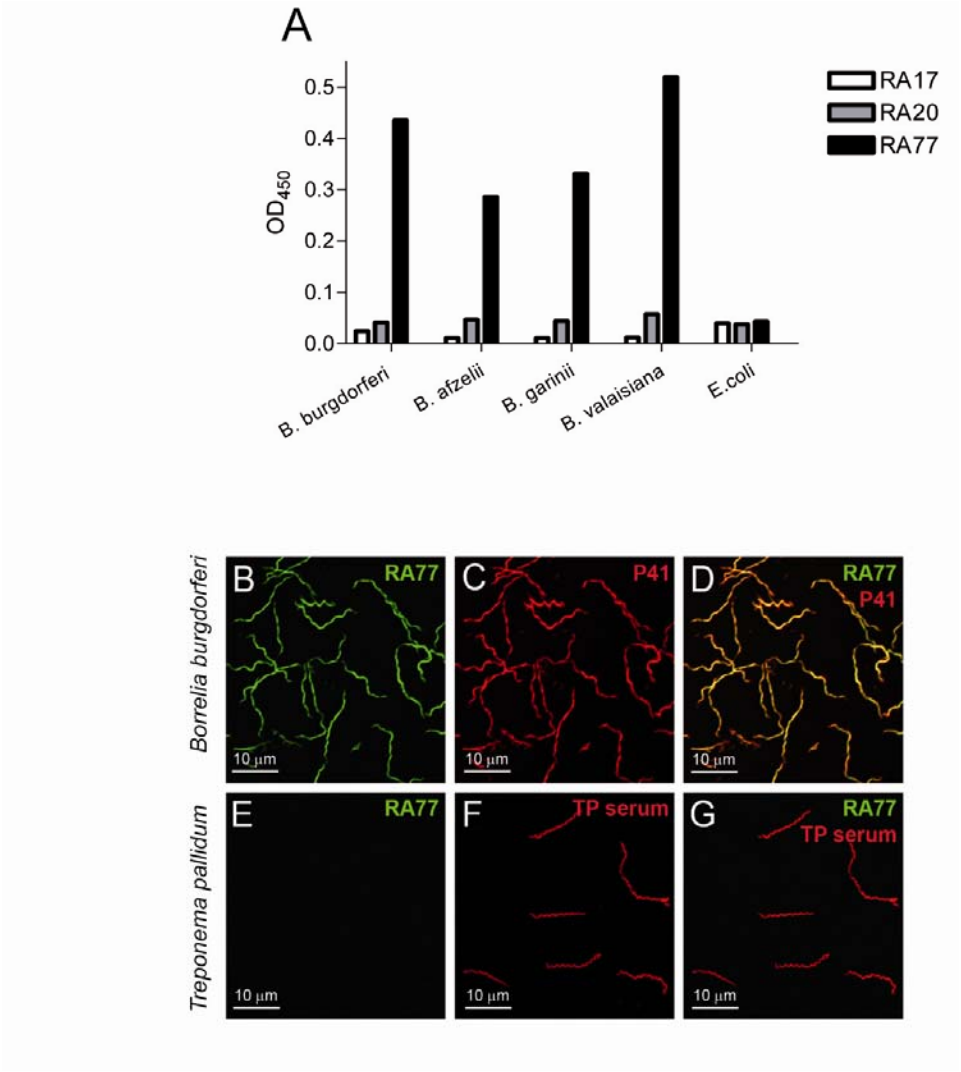

Figure 9: Specificity of RA77 for Bb without cross-reactivity to *E.coli* and Tp. **A.** ELISA coated with 5ug/ml of either Borrelia lysate of different Borrelia strains or *E.coli* lysate. Tested recombinant mAbs RA17, RA20, RA77 were used at a concentration of 10ug/ml. Staining of Bb attached to polycarbonate slide with: **B.** RA77 **C.** anti-p41 antibody 1C11 **D.** Overlay of B and C. Staining of Tp attached to polycarbonate slide with: **E.** RA77 **F.** anti-TP serum. **G.** Overlay of E and F.

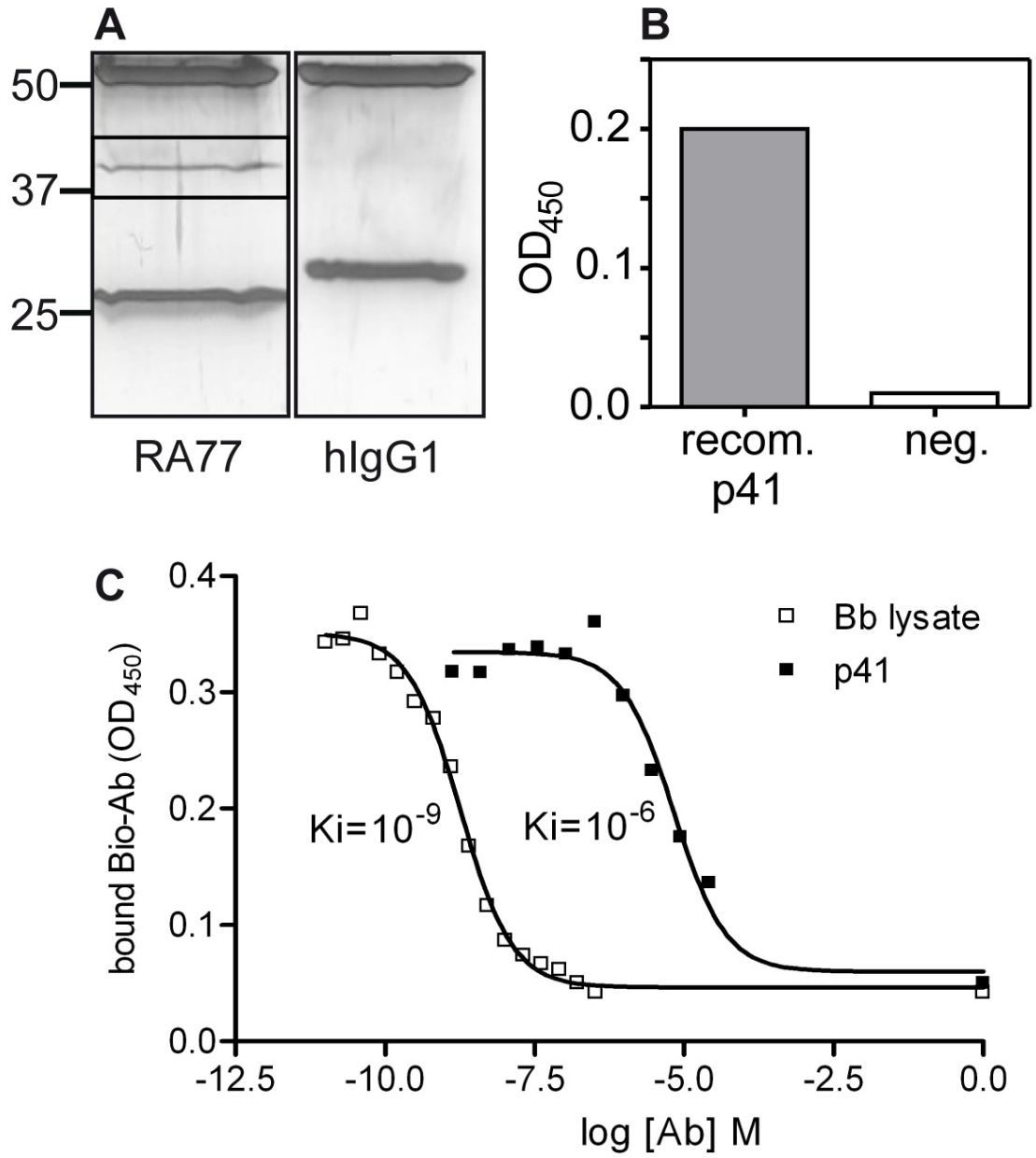

Figure 10: Bb-specific mAb RA77 recognizes p41 flagellin protein. **A.** Immunoprecipitation of p41 flagellin protein from Bb lysate by RA77. The ~40kDa band corresponds to the p41 flagellin protein (framed), as verified by MALDI TOF analysis. No such band was detected in immunoprecipitation with hIgG1. The protein bands around 50 kDa and 25 kDa correspond to the Ig H and L chain of the RA77 and hIgG1 control antibody. Molecular mass standards are indicated on the left. **B.** Verification of the specificity of RA77 for p41. Shown are the results of an ELISA experiment with RA77 binding to recombinant p41 and BSA (neg. control). **C.** Different binding affinity of RA77 to native Bb lysate and recombinant p41. Shown are the result of competition ELISAs of biotinylated vs. non-biotinylated RA77 for their binding to Bb lysate (□, Ki=10^{-9}) and recombinant p41 (■, Ki=10^{-6}).

2.2.3 Recombinant antibody RA17 is specific for auto-antigens in CNS myelin

Immunofluorescence stainings of cryostat sections of frozen human facial nerve tissue showed reactivity of RA17 with the myelin component of the nerve, as identified by co-stainings with the myelin specific antibody 8-18c5 (Fig. 11F-G). Furthermore immunofluorescence stainings on human white matter tissue revealed a specificity of RA17 for structures stained with the myelin antibody 8-18c5 (Fig. 11A, D). The structures stained by RA17 enfold neurons, as demonstrated by double immunofluorescence staining with a neurofilament specific antibody, thus indirectly confirming the myelin reactivity of RA17 (Fig. 11E). In contrast Borrelia specific RA77 antibody, antibody RA20 and human IgG1 isotype control didn't show any reactivity with human CNS tissue (Fig. 11B-C). None of the antibodies examined (RA17, RA20, RA77) stained (Fig. 11H-K), kidney and lung tissue controls (not shown).

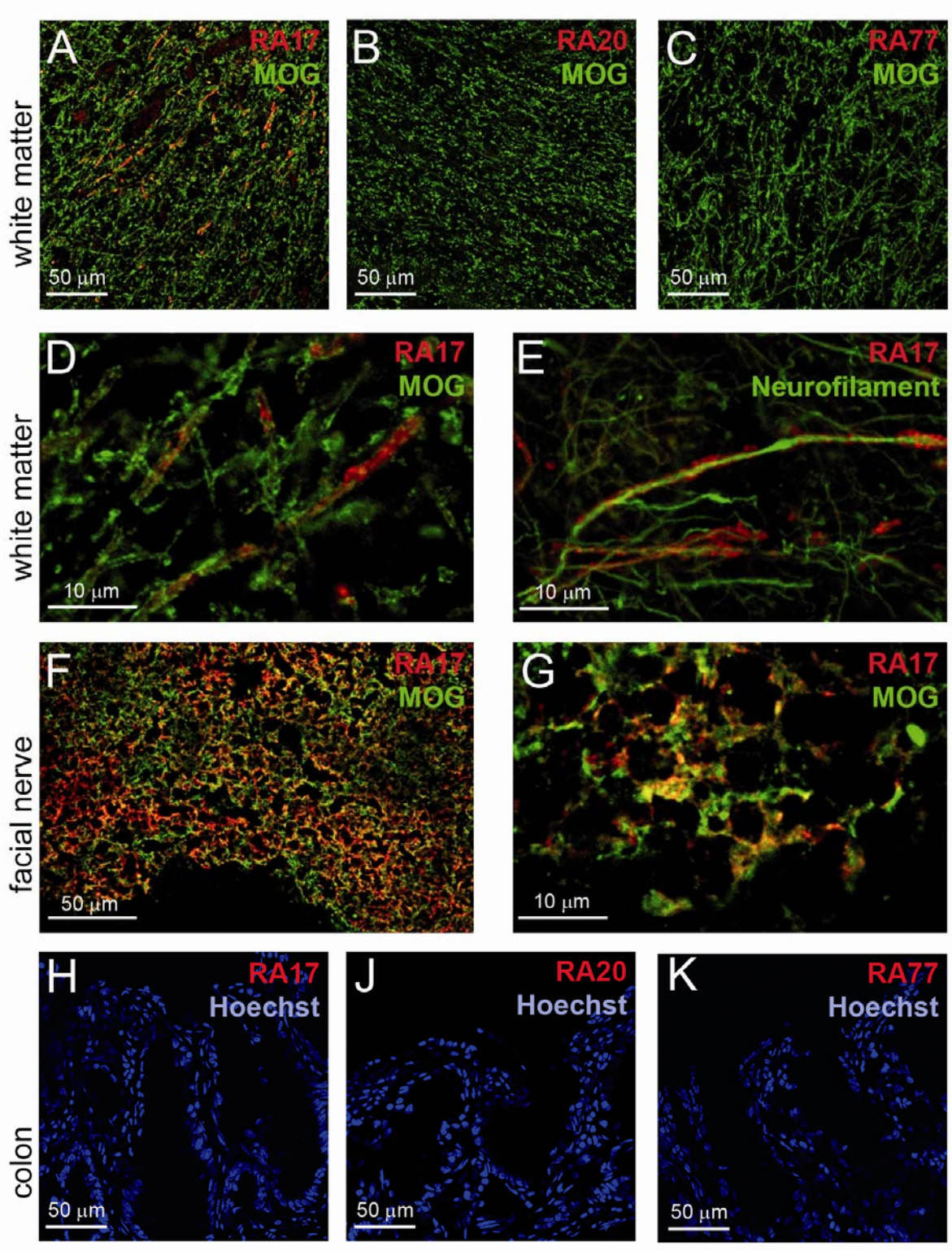

Figure 11: CNS reactivity of RA17. Micrographs of immunofluorescence stainings of human white matter (**A-E**), facial nerve (**F-G**) and colon tissue (**H-K**). White matter stainings performed with myelin-specific antibody 8-18c5 (**A-D**) and RA17 (**A**), RA20 (**B**), RA77 (**C**). **D.** shows a greater magnification of A. **E.** shows staining of white matter tissue with neurofilament-specific antibody and RA17. Stainings of human facial nerve tissue with **F.** myelin-specific antibody 8-18c5 and RA17. **G.** shows a greater magnification of F. Stainings of human colon tissue with **H.** RA17. **J.** RA20. **K.** RA77. Nuclei are stained with Hoechst 55432 (blue).

2.3 Characterization of recombinant antibodies derived from clonally expanded CSF plasma cells from MS patients

There is strong evidence that B cells and antibodies play an important role in the immune pathogenesis of MS (Link 1978). OCB are a hallmark of MS CSF (Tourtellotte et al. 1980) indicating the presence of an antigen driven B cell response in the CSF. However, little is known about the immunopathological role of the cePC and the target antigens of their antibodies remain to be determined.

We therefore produced recombinant human mAb from CSF derived cePC from four patients with early or definite MS. Table 2 shows the frequencies of the expanded plasma cells and the characteristics of the corresponding V-H and V-L sequences.

Using immunofluorescence stainings of human MS lesion tissue, my colleague Melanie Harrer could show, that seven of these antibodies (RRMS1-36, CIS1-40, CIS1-86, CIS1-92, RRMS2-34, RRMS2-101, SPMS1-61) specifically recognize myelin while the remaining two antibodies (RRMS1-6, RRMS2-106) displayed astroglial reactivity.

			Closest Germline				
Clone	Frequency		V segment	% Homology	J segment	D segment	CDR3
CIS1-40	2/21	H chain:	IGHV4-31	93.6	IGHJ5	IGHD3-22	ARNPVTDYSTGYLAWGPKNDRAWERKWFDS
		L chain:	IGKV1-12		IGKJ2		QQANNFPYT
CIS1-86	5/21	H chain:	IGHV4-b	93	IGHJ4	IGHD2-8	ARGAVFQARGTAFDL
		L chain:	IGKV1-5		IGKJ2		QQYNSYSNT
CIS1-92	2/21	H chain:	IGHV3-7	96.9	IGHJ3	IGHD6-19	ATYPNRITVAGFDAFEI
		L chain:	IGKV1-9		IGKJ4		QQLHSYPLT
RRMS1-6	13/57	H chain:	IGHV4-4	87.3	IGHJ6	IGHD1-26	ARGLSGSPVLRRNPLPGGYYYGMDV
		L chain:	IGKV1-39		IGKJ4		QQSYSSPLT
RRMS1-36	4/57	H chain:	IGHV3-7	92.5	IGHJ6	IGHD1-1	ARDRFWHVQLELDHPSGMAV
		L chain:	IGKV1D-17		IGKJ1		LQHKIYPPT
RRMS2-34	2/66	H chain:	IGHV3-33	94.4	IGHJ3	IGHD2-15	ARDKTVGYCSAGSCYGTTDALDI
		L chain:	IGKV1-5		IGKJ1		QQYDTYPWT
RRMS2-101	4/66	H chain:	IGHV1-18	92.4	IGHJ6	IGHD3-10	ARGVAANYYYYGMDVW
		L chain:	IGKV1D-33		IGKJ1		QRYDDLPTWS
RRMS2-106	4/66	H chain:	IGHV3-21	91.9	IGHJ6	IGHD3-16	ARDRVPFELRFYGMDV
		L chain:	IGKV3D-15		IGKJ2		QQYSHWGHT
SPMS1-61	4/24	H chain:	IGHV1-69	93.1	IGHJ6	IGHD2-15	ATCTSCTGGSCYHKCNYYHGMDV
		L chain:	IGKV1-47		IGKJ3		ATWDDNLSGPV

Table 2: Characteristics of VH and VL region sequences of recombinant antibodies derived from expanded CD138+ CSF plasma cells from MS patients. Frequency indicates the number of the

respective H chain sequences containing the indicated H chain CDR3 region among all plasma cells analyzed by PCR. Shown are the most homologous V, D and J germline segments, degree of homology to the closest germline segment for the H chain V region and the respective CDR3 amino acid sequence.

2.3.1 Human brain cDNA expression library

To investigate the cognate antigens of the recombinant MS-mAb, we applied a novel protein array, which has already been used to delineate antigen specificities of CSF Ig derived from MS patients (Cepok et al. 2005). We conducted these array experiments prior to the immunofluorescence staining experiments mentioned above. The protein array was generated from a human fetal brain cDNA expression library comprising 37'000 bacterial expression clones. We conducted several of these array experiments with either individual MS-mAb or mixes of MS-mAb. Immunoreactivity was visualized using an HRP-conjugated anti-human IgG. Between 10 and 74 expression clones with a specific staining above background were identified on arrays incubated with individual MS-mAbs (mAb-RRMS1-6: 74, mAb-CIS1-40: 10, mAb-RRMS2-106: 21). Figure 12 shows an example of antibody binding to a expression clone spotted in duplicate on the array. A total of 66 of these arrayed expression clones were ordered, subsequently their cDNA inserts were sequenced and the corresponding AA sequences were determined. From several other clones, sequence information was obtained from the RZPD (German Resource Center for Genom Research), the supplier of the protein-arrays used.

In several of these positively stained clones the transgene was expressed in a wrong reading frame generating artificial protein sequences.

All the ordered clones, irrespective of whether they were expressed in the correct frame or not, were purified and ELISA experiments were conducted. Unexpectedly, no clear specificity for any of the MS-mAb could be detected in these ELISA experiments. However, some MS-mAbs interacted with certain proteins, but only at a high concentration of 10ug/ml but not with 3ug/ml. Nevertheless, low affinity interactions cannot be excluded. Table 3 summarizes the possible antigens delineated from the protein-arrays and subsequent ELISA experiments.

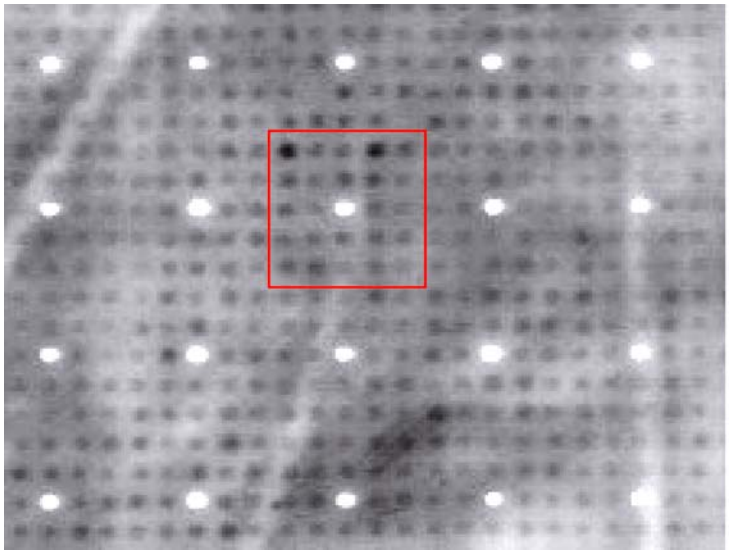

Figure 12: Part of the makroarray comprising bacterial expression clones probed with MS-mAb. Positive binding is visualized with anti human IgG-HRP and fluorescence substrate. Example for positive binding to clones spotted in duplicate is framed in red.

mAb	RZPD Clone	Clone ID	Protein-Blast
mAb-RRMS1-6	1	H08523	Vimentin
	4	F02536	out of frame
	9	O24552	Vimentin
	23	H06528	out of frame
	39	H20557	Sequestosome 1 (SQSTM1)
mAb-CIS1-40	4	F02536	out of frame
	22	E09526	Debrin like protein (DBNL)
	58	C18590	Ufm 1-conjugating enzyme 1 (UFC1)
	63	F16598	Lipidosin, MP-binding enzyme (ACSBG1)
mAb-CIS1-86	4	F02536	out of frame
	22	E09526	Debrin like protein (DBNL)
	63	F16598	Lipidosin, MP-binding enzyme (ACSBG1)
mAb-RRMS2-106	4	F02536	out of frame
	9	O24552	Vimentin

Table 3: Possible interaction partners delineated from protein macroarrays and subsequent ELISA experiments.

2.3.2 Antibodies produced by cePC from MS CSF do not react with MOG or MBP

A number of published studies have shown that antibodies specific for MOG and MBP are present in the serum and CSF of MS patients (Karni et al. 1999; Lindert et al. 1999; Mantegazza et al. 2004; Markovic et al. 2003; Reindl et al. 1999; Sun et al. 1991; Xiao et al. 1991). We therefore evaluated if any of our nine MS-mAbs is reactive with one of these two myelin antigens. We performed ELISA experiments with the recombinant extracellular domain of human MOG produced in *E.coli* and with purified human MBP. No reactivity could be demonstrated for any of the tested antibodies (Fig. 13A). As it has been shown that antibodies against glycosylated epitopes of MOG possess myelin destructive potential (Marta et al. 2005) we also tested our antibodies in FACS analysis with eukaryotic cells transfected to express native human MOG on their surface (Fig. 13B). As was the case for the ELISAs, no reactivity of any of the MS-mAb could be detected.

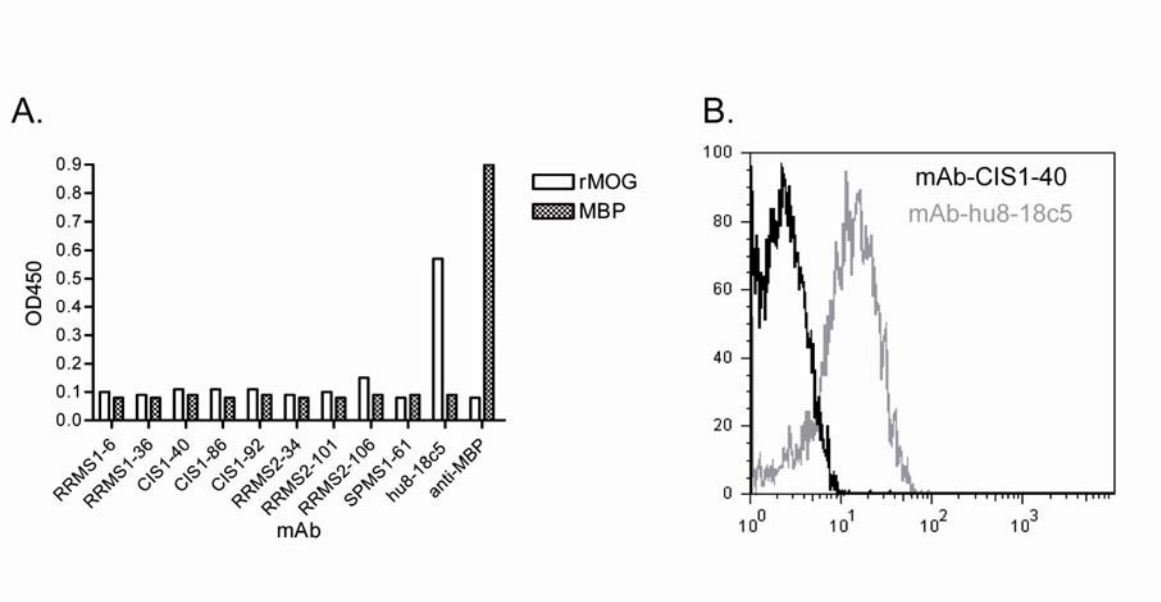

Figure 13: mAbs derived from cePC do not react with MOG or MBP. Panel A shows ELISA experiments demonstrating the lack of reactivity with recombinant MOG or purified human MBP. Panel B shows histograms of FACS experiments with a eukaryotic cell line (Jurkat) transfected to express human MOG on its surface. Note the clear reactivity (shift to the right) of humanized anti-MOG 8-18c5 antibody (light grey histogram). In comparison no reactivity of cePC derived MS-mAbs (here: CIS1-40, black; identical findings for all other MS-mAbs, not shown) with MOG-expressing Jurkat cells could be demonstrated.

2.3.3 Antibodies produced by cePC from MS CSF do not react with human myelin in western blot and FACS experiments

As outlined above, seven of nine MS CSF derived antibodies (RRMS1-36, CIS1-40, CIS1-86, CIS1-92, RRMS2-34, RRMS2-101, SPMS1-61) showed a myelin specific immunofluorescence staining on MS lesion tissue. To confirm these findings, we performed further experiments using myelin extracted from frozen human MS and healthy control brain tissue.

First we performed extensive western blot analysis on large gradient acrylamide gels. Figure 14A shows a western blot with different control antibodies confirming a proper myelin isolation and western blot experimental set-up. However, no band additional to the bands from secondary antibody only could be detected with any of the myelin specific MS-mAb (Fig. 14B).

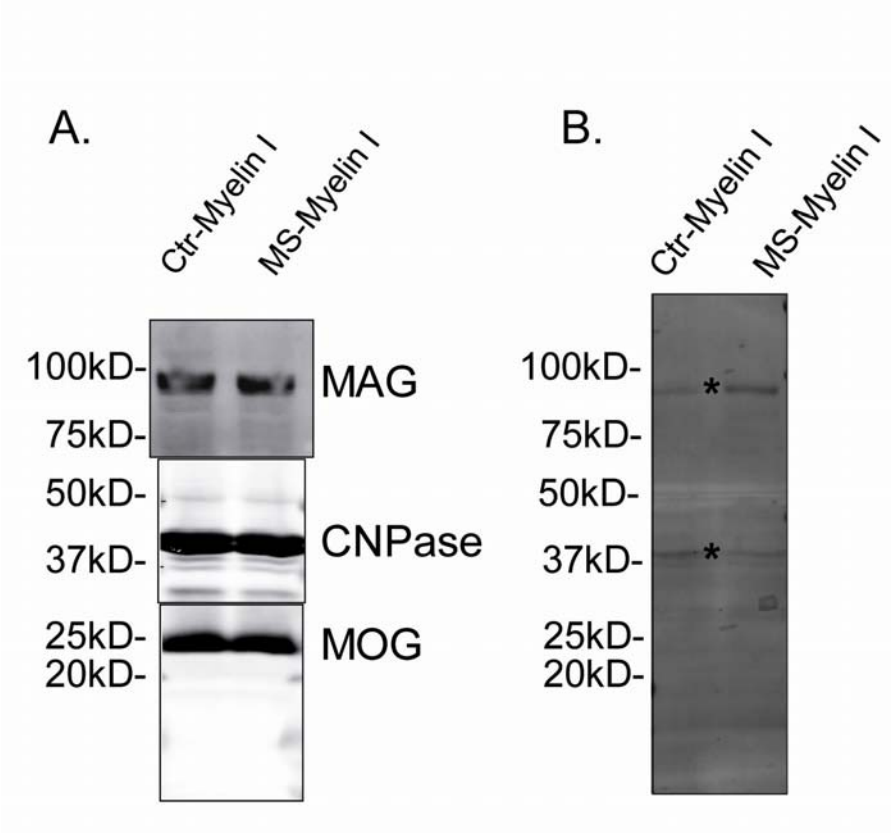

Figure 14: Western blot analysis of human myelin extracted from MS and control brain tissue. A. The myelin preparations were tested with antibodies specific for myelin associated glycoprotein (MAG), cyclic 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNPase) and myelin ologodendrocyte glycoprotein (MOG). B. Myelin western blot probed with MS-mAb RRMS2-34. Bands marked with asterisks were also present with secondary antibody only (data not shown).

There are different possible reasons why these western blot experiments were not successful. One possible explanation is that under the denaturating conditions of western blot analysis potential epitopes were destroyed. To overcome this problem we performed myelin FACS experiments in which the myelin proteins should be in their native conformation with all posttranslational modifications. As myelin consists of more than 70% lipids, it forms small vesicles if dissolved in a physiological buffer. Figure 15 shows FACS stainings of such small myelin vesicles. The only antibody demonstrating clear reactivity for the vesicles was MS-mAb RRMS1-6, one of the antibodies displaying an astrocyte like immunofluorescence-staining.

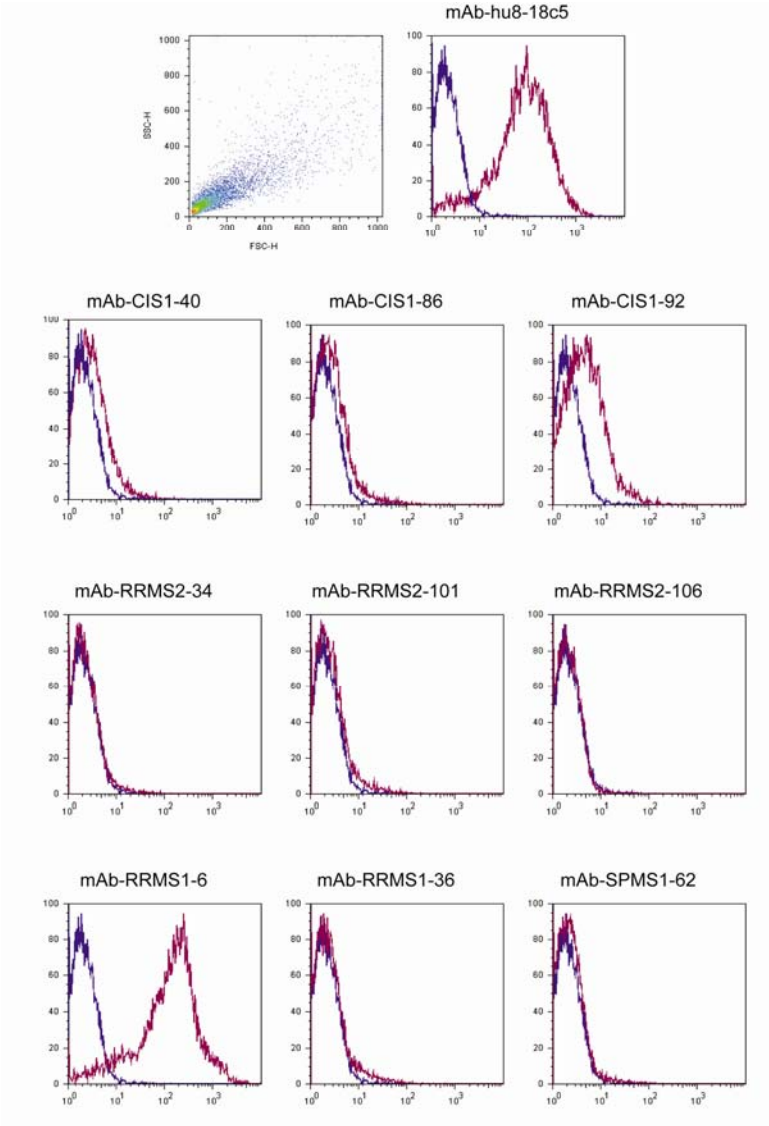

Figure 15: FACS analysis of myelin-vesicles stained with MS-mAb. Forward and side-scatter analysis of the vesicles is shown in the upper left panel. The red line in the histograms shows staining of the respective mAb, while the blue line represents the isotype control staining. Positive control anti-MOG hu8-18c5 shows clear binding. However, from the MS-mAb only mAb-RRMS1-6 binds strongly to the myelin-vesicles. X-axis of the histogram shows fluorescence intensity, Y-axis percent of cell counts.

3 ORIGINAL RESEARCH ARTICLES

Pathogen specificity and autoimmunity are distinct features of antigen driven immune responses in neuroborreliosis

Sandra Kuenzle*, Hans-Christian von Büdingen*, Mirjam Meier, Melanie D. Harrer, Eduard Urich, Burkhard Becher and Norbert Goebels

* SK and HCVB contributed equally

Infection and Immunity, August 2007, in press.

Abstract:

Neuroborreliosis (NB) is a chronic infectious disease of the central nervous system caused by a tick borne spirochete, *Borrelia burgdorferi* (Bb). In addition to direct effects of the causative infectious agent, additional immune mediated mechanisms are thought to play a role in the central nervous system (CNS) pathology of NB. In order to further understand the involvement of humoral immune mechanisms in NB, we dissected the intrathecal antibody responses down to the single plasma cell level. Starting with single cell RT-PCR of FACS sorted cerebrospinal fluid plasma cells from a NB patient, we identified expanded clones and resurrected the antigen specificity of their secreted antibodies through recombinant expression of the correctly paired Ig heavy and light chain genes as monoclonal antibodies (mAb). As expected, we found specificity for the causative infectious agent Bb among the clonally expanded plasma cell (cePC) derived mAb. However, from an independent cePC of the same patient, we could derive mAbs specific for human CNS myelin, without detectable crossreactivity with Bb antigens. While reactivity against Bb is a known feature of humoral immune responses in NB, we show a) that immune responses specific for self-antigens may be a distinct feature of CNS infections independent of pathogen reactivity and b) that humoral autoimmunity in NB – since found in cePC - is the result of a truly antigen driven immune response. Our findings indicate that in NB mechanisms may be at play that induce distinct immune responses specific for pathogen and self-antigens independent from “molecular mimicry”.

Thesis author work: This manuscript is the thesis core and experiments were mainly performed by myself. Contributions from other people comprise part of the immunofluorescence experiments and help in preparing the manuscript.

Clonally expanded plasma cells in the CSF of MS patients produce CNS specific immunoglobulins

Hans-Christian von Büdingen[#], Melanie D. Harrer[#], **Sandra Kuenzle[#]**, Mirjam Meier, Tobias A. Rupprecht and Norbert Goebels

[#] HCVB, MH and SK contributed equally

Submitted.

Abstract:

Clonally expanded plasma cells (cePC) and their presumed products, oligoclonal immunoglobulin bands (OCB), are characteristic findings in the cerebrospinal fluid (CSF) of patients with multiple sclerosis (MS). While cePC and OCB strongly suggest an involvement of B cell dependent immune mechanisms in the pathogenesis of MS, their actual pathological relevance and target antigens remain unknown. To further understand the potential role played by cePC we generated a panel of monoclonal recombinant antibodies (MS-mAb) from MS CSF-derived cePC from 4 patients with early or definite MS. Single cell RT-PCR of correctly paired heavy and light chain immunoglobulin genes from individual cePC ensured the subsequent resurrection of their original antigen specificity. Immunofluorescent stainings of MS lesion tissue with MS-mAbs revealed myelin reactivity in the cePC repertoire of all 4 patients and astroglial reactivity in 2 patients. Our findings provide conclusive evidence for the presence of an antigen driven B cell response culminating in the expression of CNS specific antibodies in the CSF of MS patients.

Thesis author work: I produced some of the MS-mAb. Furthermore I performed the FACS experiments.

Exploiting the tumor-specific immune response of cancer patients for the isolation of tumor antigen specific human monoclonal antibodies

Christoph Esslinger*, **Sandra Kuenzle***, Irene Abela*, Alfred Zippelius, Dirk Jäger, Alexander Knuth, Roger M. Nitsch*, Holger Moch*, and Norbert Goebels*

*CE, SK and IA as well as RMN, HM and NG contributed equally

Submitted.

Abstract:

Antibodies specific for tumor-associated antigens have been identified in the sera of a variety of cancer patients. Their clinical significance however, was difficult to assess due to the lack of a straightforward approach for the isolation and cloning of patient-derived antibodies. In the present study we describe a method for the isolation, molecular cloning and recombinant production of patient-derived human antibodies to tumor-associated antigens. This was achieved by screening of oligoclonal memory B cell cultures established from patient PBL combined with a molecular cloning step using single cell RT-PCR and re-screening of recombinant antibody clones.

In a proof of principle study we cloned a human antibody specific for the tumor-associated antigen NY-ESO-1 from a melanoma patient who was seropositive for NY-ESO-1 in ELISA and on autologous tumor sections. The screening was performed on ELISA and on tumor tissue using an adaptation of the tissue micro array technology. The obtained tissue-reactive human monoclonal antibody was shown to bind to the N-terminus of NY-ESO-1 that is also shared by the tumor-associated antigen LAGE-1.

This method greatly facilitates the cloning of patient-derived antibodies; applied to selected clinical responders it may lead to the identification and isolation of novel candidate antibodies for the immunotherapy of cancer.

Thesis author work: I performed the single cell PCR, cloning and recombinant production of the tumor specific monoclonal antibody. Furthermore I performed the epitope mapping, the avidity and the immunofluorescence experiments.

4 DISCUSSION

4.1 Recombinant antibodies: general considerations

Antibodies play a central role in adaptive mammalian immunity against invading pathogens but if erroneously directed against self, they can also lead to and/or support autoimmune processes. The key feature of antibodies is their ability to bind with high specificity and affinity to their cognate antigen. Antibodies can scavenge their target by forming immune-complexes, mediating phagocytosis or recruiting cytotoxic systems.

To date, little is known about the role played by antibodies in the CSF of patients with neuroinflammatory diseases. The analysis of the antigen specificity and disease relevance of antibody responses in the CSF has been hampered by both the limited availability and oligo/polyclonal composition of CSF Ig. Furthermore, CSF contains both intrathecally synthesized oligoclonal IgG and blood-derived IgG. However, antibodies derived from cePC in the CSF are particularly expected to be disease relevant. Our goal was therefore to establish a method to accurately dissect the various antigen specificities contained in CSF down to a clonal level and to resurrect them in the form of recombinant mAbs. The availability of mAbs would thus provide us with appropriate tools for the characterization of both cognate target antigen(s) and functional relevance.

Several technologies have enabled the cloning and isolation of human antibodies including different phage display technologies. Such technologies are based on random combinatorial pairing of the variable region genes of Ig H and L chains. This dissociation of the natural H and L chain pairing may destroy the original specificity and may lead to unwanted new specificities. It is therefore important to implement methods starting from single antibody producing cells and resurrect their original specificity.

More than 30 years ago, Köhler and Milstein invented the technology for producing mAb by immortalizing murine B cells, which earned them the Nobel prize (Kohler and Milstein 1975). Alternative techniques for human B cell cloning have been developed, such as EBV transformation (Traggiai et al. 2004) or single B cell culture (de Wildt et al. 1997; Weitkamp et al. 2003). However, these methods mostly have relatively low

cloning efficiencies, are difficult to perform with human B cells and have not been successfully used with plasma cells. Therefore they are unsuitable for use when handling the limited amount of B cells in the CSF and are clearly inadequate for single human plasma cells.

Following experimental procedures published by others (Owens et al. 2004; Wardemann et al. 2003), we established a system to amplify the paired rearranged Ig H and L chain genes of individual plasma cells from CSF by single cell RT-PCR. Cloning of these amplicons into expression vectors and the use of an eukaryotic expression system permitted the production of recombinant human mAb representing the original specificity of the isolated single plasma cells.

In pilot experiments we validated our system. We therefore amplified Ig H and L chain V region genes from a hybridoma producing the MOG-specific antibody 8-18c5. We cloned these V regions into the same expression system used for plasma cell derived antibodies and produced a chimeric hu8-18c5. Competition ELISA experiments with the original 8-18c5 and the recombinant hu8-18c5 demonstrated that both specificity and affinity are preserved by recombinant expression.

The methods described herein open new routes for the characterization of plasma cell responses in inflammatory CNS disease. They may facilitate the identification of potentially disease relevant target antigens recognized by antibodies in the CSF of patients with inflammatory CNS diseases.

Additionally, this experimental strategy may be used to exploit the focused human immune response to generate human mAbs of both pathogen and auto-antigen specificity, which might be used as therapeutic agents. Since such antibodies are matured in the human immune system they may bear advantages of lower immunogenicity and “human-like” specificity as compared to mAbs produced in mice.

Combining the described resurrection of mAb and the cultivation of peripheral memory B cells from a cancer patient, we succeeded in the production of a human tumor specific antibody (described in Esslinger, Kuenzle *et al.* submitted). To our knowledge, this is the first patient derived tumor specific mAb. The application of this technique to selected clinical responders may lead to the identification and isolation of novel candidate antibodies for the immunotherapy of cancer.

4.2 Clonally expanded plasma cells

The clonal expansion of B cells and their differentiation into plasma cells is a characteristic feature of an antigen driven immune response. All members of a plasma cell clone bear the same or highly similar Ig sequences as they have developed from a single proliferating ancestor B cell. In inflammatory CNS diseases, cePC are believed to be the producers of OCB. In infectious CNS diseases, OCB have been demonstrated to include specificities to the causative agent (reviewed in (Gilden et al. 1996)). Despite the fact that non-clonally expanded plasma cells may also produce disease relevant antibodies, as demonstrated by Burgoon et al in SSPE (Burgoon et al. 2006), the present study is focused on investigating the humoral immune response as expressed by cePC because cePC undoubtedly represent an active part of an antigen specific immune response.

CePCs and clonally restricted Ig gene usage can be found in MS CSF and lesion tissue cells, respectively (Baranzini et al. 1999; Ritchie et al. 2004). In the present study the cePCs were identified as a group of plasma cells bearing identical CDR3 region sequences.

The IGHV segments of all cePC derived H chain sequences contained several mutations as compared to their most closely related germline sequences, providing evidence that affinity maturation including somatic hypermutation has occurred in these antibodies.

Some groups of H chain V regions sharing identical H-CDR3s showed infrequently interspersed replacement mutations. The finding of such clonal variants in the CSF could be a hint that the CSF/CNS is an environment playing a supporting role for affinity maturation in antigen driven B cell responses. Other groups of sequences from cePC did not show such replacement mutations but bored Ig gene sequences of 100% identity. Since a number of precautions had been taken to avoid cross contamination between wells of PCR plates (see Materials and Methods), and we also obtained several "unique" sequences from single plasma cells analyzed in the same PCR experiment, we expect that sequences of 100% homology are not a product of contamination. Also the fact that we obtained identical Ig L chain sequences from the plasma cells bearing identical Ig H chain sequences make a “dual” cross-contamination extremely unlikely.

4.3 Recombinant antibodies from clonally expanded CSF plasma cells from a neuroborreliosis patient

4.3.1 Borrelia specific antibody mAb-RA77

4.3.1.1 Proof of principle

One (RA77) of the human mAbs derived from cePCs in the CSF of a patient with NB specifically reacted with Bb, the causative infectious agent of NB. ELISA experiments with different Bb strains demonstrated that mAb-RA77 binds to all tested Bb strains and does not cross-react with *E.coli*. Immunofluorescence stainings of Bb confirmed Bb specificity of mAb-RA77. To exclude cross-reactivity of mAb-RA77 with other spirochete bacteria we also evaluated possible binding to Tp. No binding to Tp could be detected again confirming Bb-specificity of mAb-RA77.

This finding of a Bb specific antibody produced by a cePC is consistent with previous studies showing that OCB in the CSF of NB patients contain antibodies directed against Bb (Hansen et al. 1990; Martin et al. 1988a). This finding is also consistent with recent data from our laboratory confirming that cePC are in fact the producers of OCB (unpublished data). To date, the only other inflammatory disease for which a link between antibodies expressed by CNS derived plasma cells and the causative agent has been demonstrated is SSPE: Burgoon et al. obtained measles virus-specific antibodies from individual plasma cells excised from post mortem brain tissue of a patient with SSPE by laser capture microdissection (Burgoon et al. 2005).

In contrast to infectious CNS disease, the antigen specificity and pathogenic role of OCB in presumed autoimmune conditions such as MS remains to be defined.

The successful generation of the pathogen specific mAb-RA77 from a single CSF plasma cell demonstrates that the method described here accurately resurrects the antigen specificity of the original plasma cell. It validates the utility of the described technical approach for the characterization of plasma cell responses in inflammatory diseases.

This proof of principle was important as we also applied this approach for investigating the unknown origin of the humoral immune response in MS. Exploiting the described technique could lead to the identification of disease relevant target antigens in MS. First steps toward this goal are described in section 2.3 and in von Büdingen, Harrer, Kuenzle *et al.*, submitted.

4.3.1.2 Further characterization of mAb-RA77

To further analyze the exact antigen specificity of the Bb reactive RA77 mAb, We performed immunoprecipitation and subsequent MALDI-TOF analysis. We were thereby able to identify the cognate antigen of RA77, namely the Bb flagellum protein p41. While We found a high affinity interaction between antibody RA77 and native Bb lysate, its affinity to recombinant p41 protein was significantly lower. This finding suggests that the interaction between RA77 and native p41 may not only depend on its amino acid sequence, but also on conformation and potential posttranslational modifications. Such modifications have been demonstrated in flagellum proteins of other spirochetes (Brahamsha and Greenberg 1988; Li et al. 1993; Wyss 1998). Another explanation for the lower affinity of antibody RA77 for the recombinant p41 protein as compared with the native protein may be partial misfolding of the recombinant protein. Nevertheless, the high affinity interaction between RA77 and native Bb lysate suggests that RA77 is the result of an affinity matured antibody response.

4.3.2 Myelin specific antibody mAb-RA17

Besides the immune response to Bb, analysis of the immune response in the CSF of patients with NB has also revealed auto-antibodies to CNS proteins (Kaiser 1995) and T cells recognizing peptides derived from CNS specific auto-antigens (Hemmer et al. 1999; Lunemann et al. 2006). Such auto-aggressive immune cells could contribute to the CNS tissue damage observed in some NB patients. We tested the cePC derived mAb for possible reactivity with auto-antigens. Because the patient, whose cePC were used in this study, suffered from facial nerve palsy, a common clinical presentation of NB, we first investigated a possible specificity of RA17, RA20 and RA77 for facial nerve tissue.

Interestingly, one of these antibodies, RA17 displayed reactivity with this tissue. To further evaluate the tissue components RA17 is specific for, we performed double immunofluorescence stainings with a myelin specific antibody (8-18c5), which strongly suggested myelin reactivity of RA17. Although not completely overlapping, co-staining of the 8-18c5 antibody and RA17 indicated reactivity with identical

structures, representing myelin. However, myelin reactivity of RA17 was not limited to facial nerve tissue but could also be detected in CNS white matter myelin. Double stainings with RA17 and a neurofilament specific antibody indirectly confirmed this notion, as RA17 stained structures enfolding neurons. Immunofluorescence stainings with RA17 on a number of different control human tissues (colon, kidney, lung) yielded negative results, suggesting CNS restricted reactivity.
Whether the appearance of the RA17 mAb actually bears immunopathological significance will be the subject of further studies. However, as diffuse white matter lesions are frequently observed in NB there might be a contribution of such auto-reactive antibodies to myelin damage.

4.3.3 Distinct immune responses – no molecular mimicry

Our findings suggest that aside from pathogen directed humoral immune responses, immunopathological mechanisms occurring in CNS infectious disease may also evoke autoimmunity. Various Bb specific antibodies cross-reacting with CNS auto-antigens have been described (Alaedini and Latov 2005; Dai et al. 1993). These studies support the hypothesis that autoimmunity due to molecular mimicry may play a role in NB. Hypothetically, molecular mimicry mechanisms would be due to Borrelia-specific epitopes shared with human tissue and are known to be important in the progression of Lyme arthritis (Gross et al. 1998). However, as neither Borrelia specific mAb RA77 reacts with human tissue nor auto-reactive RA17 with Borrelia no such molecular mimicry mechanisms could be described in the present study. We rather provide evidence that in NB additional mechanisms, eliciting distinct immune responses for pathogen and self-antigens may be at play.
This novel finding could be delineated by the use of the described single cell approach. As we reconstructed the antigen specificities of antibodies secreted by individual expanded CSF plasma cell clones, we were able to dissect the complex repertoires of the adaptive humoral immune system in response to CNS infection with Bb.
Interestingly, not only the Bb specific but also the auto-reactive antibodies were originally produced by cePC. This demonstrates that in NB humoral autoimmunity can also be the result of a truly antigen driven immune response. However, as

mentioned above, the immunopathological relevance of such auto-reactive antibodies remains still elusive.

4.4 Recombinant antibodies from clonally expanded CSF plasma cells from MS patients

OCB are a hallmark feature of MS CSF but their pathological role still remains elusive. A first step towards a better understanding of immune responses in MS would be the identification of the specificity of these Ig.

A number of studies have investigated the Ig in the CSF of MS patients; among others, reactivity to the auto-antigen MOG (Xiao et al. 1991) and to viral proteins of EBV (Cepok et al. 2005) have been described. Focusing on the antigen driven immune responses, other investigators have found an axon-directed antibody response by analyzing recombinant antibodies constructed from CSF B cell clones (Zhang et al. 2005) and OCB specific for Chlamydia pneunomia have also been described (Franciotta et al. 2005).

By applying the herein described and validated approach for the production of mAbs from cePC, we produced nine recombinant mAbs from plasma cell clones in the CSF of four MS patients. To determine their cognate antigen we applied a number of experimental approaches. A rather large effort was undertaken using clones of a human fetal brain cDNA expression library spotted on nitrocellulose membranes. Incubation of this library with our MS-mAbs resulted in a number of potential target antigens, which when tested in purified form did not turn out to be high affinity targets, suggesting that these findings are rather due to non-specific binding. There are a number of different possible explanations why this approach failed. As the proteins of this library are expressed in *E.coli*, posttranslational modifications are absent and intact conformation is not assured. However, such attributions could be important in antibody antigen complex formation. Another possible reason for the negative results of these experiments is that the library is derived from fetal human brain (*week 14 post fertilization*) cDNA. At that developmental stage, axons of the human brain have not undergone myelination, therefore myelin proteins are not yet expressed and will not be represented in this cDNA library. Because, as was later shown in immunofluorescence experiments performed by my colleague Melanie

Harrer, most of our MS-mAbs bind to myelin, a positive result from this brain cDNA library seems unlikely.

As mentioned, seven of nine MS-mAb react with CNS myelin and two with astroglia. Most prominently, the seven myelin reactive MS-mAbs bound to myelin at the rim of MS lesions. Myelin destruction is a major histopathological feature of MS and has been strongly associated with the humoral immune response. Namely, antibody and complement depositions have been described in areas of myelin destruction (Lucchinetti et al. 2000; Storch et al. 1998) and MOG-specific antibodies have been found in areas of active myelin disintegration (Genain et al. 1999). However, whether antibodies detecting myelin proteins in the serum of MS patients can be used as diagnostic or prognostic markers for MS or wheter they play a role in the pathogenesis of MS remains a matter of debate (Berger et al. 2003; Kuhle et al. 2007).

The finding of antibodies derived from MS CSF cePC actually recognizing CNS myelin indicates that an antigen driven immune response against myelin proteins takes place in the CNS compartment of MS patients. To determine the antigen specificity, an obvious first choice was to examine whether our MS-mAb displayed specificity for the commonly studied CNS myelin antigens MOG and MBP.
No reactivity with MBP (purified from human brain) or recombinant MOG could be detected in ELISA experiments. As recent research demonstrated that antibody responses against glycosylated epitopes of MOG possess myelin destructive potential (Marta et al. 2005) we also tested reactivity to eukaryotic cells (Jurkat) transfected to express native human MOG on their surface. No reactivity of any MS-mAb could be demonstrated by FACS analyses.
In a next step we performed western blotting experiments using a myelin extract made from a post mortem MS patient brain. However, no specific reactivity could be detected for any of the MS-mAb reacting with myelin in immunofluorescence experiments. Because it is known that pathogenic antibodies often recognize conformation dependent epitopes (von Budingen et al. 2004), this negative result could be due to the denaturating conditions used in western blotting. We therefore performed FACS experiment with myelin vesicles stained with our MS-mAb. As these vesicles are formed in physiological buffer myelin proteins would be expected to be

present in their native conformation with all posttranslational modifications present. In spite of this, no significant binding of MS-mAbs to myelin vesicles could be detected. The reason for the fact that only immunofluorescence experiments were able to reveal myelin specificity of MS-mAbs remains undetermined. However, it could be that the antigen is somehow omitted from the myelin preparation or not abundant enough to be detected in the techniques employed. As the immunofluorescence staining is most pronounced at the rim of MS lesions, it could be that the antigen is only expressed in such restricted areas. If this was the case, the concentration of the antigen would be very small in a whole myelin preparation.

4.4.1 Possible explanations why we failed so far in identifying the cognate antigens

Even after using various experimental approaches, so far we have been unsuccessful in the identification of the cognate antigens of the auto-reactive mAb from CSF cePC. In that context it is of interest to note that another recent study, employing random peptide libraries, also failed to identify potential target antigens of mAb derived from MS CSF cePC (Yu et al. 2006).

Several different explanations are possible for these difficulties:

1. Specificity of MS-mAbs for epitopes involving lipids: CNS lipids have been described to be target antigens of the humoral immune response in MS (Kanter et al. 2006; Menge et al. 2005). However, so far no experiments appropriate for the detection of lipid-epitopes have been conducted.

2. Specificity for epitopes involving posttranslational modifications: Post-translational modifications of proteins occur very frequently and several hundred different ones are known. The role played by these modifications is poorly understood at this time. However, post-translational modifications may determine the nature and extent of secondary and tertiary structure, permitting the protein to adopt multiple conformations and thus providing possible targets for autoimmune responses.

Recent studies established that MBP from MS brain is less phosphorylated and more methylated compared to MBP from normal brain (Kim et al. 2003). Furthermore it has been demonstrated that in MS brain MBP is more citrullinated (Moscarello et al. 1994) and the operating enzyme converting arginine to citrulline (peptidylarginine deiminase) has been shown to be upregulated (Moscarello et al. 2007). The relevance of such differences in post-translational modifications remains to be elucidated. However, serum antibodies reactive to citrullinated proteins/peptides have been shown to be a specific marker for the autoimmune disease rheumatoid arthritis (Migliorini et al. 2005). It is possible that in the approaches we used to investigate the antigen(s) recognized by our mAbs, such novel autoimmune epitopes generated by particular post-translational modifications were absent.

3. Specificity for polysaccharide and/or carbohydrate moieties: Another possibility would be that our mAbs specifically recognize molecules with glycosidic bonds involving sugars (polysaccharides or carbohydrates), so called glycans. Recent studies have demonstrated increased serum anti-Glc(alpha1,4)Glc(alpha) (Schwarz et al. 2006) and anti-N-glycosylated peptide (Lolli et al. 2005) antibodies in relapsing-remitting MS patients. The latter study could even show via immunohistochemistry of brain tissue, that these anti-N-glycosylated peptide antibodies recognize myelin and oligodendrocyte auto-antigens. Though most of these anti-glycan antibodies are of IgM isotype, it would be interesting to test whether our MS-mAb obtained from IgG producing cePC possibly recognize such epitopes.

4. Specificity for viruses – molecular mimicry: Viruses have been associated with MS for many years and several viruses have been isolated from brains of MS patients (Fujinami et al. 2006). Recent work suggests a humoral immune response directed against EBV to be a dominant feature of OCB (Cepok et al. 2005). Thus, we had our MS-mAbs tested for reactivity against seven common candidate viruses (EBV, CMV, HSV, HHV-6, measles, rubella, VZV), yielding negative results. However, it is possible that our MS-mAbs are targeting so far unknown viral agents underlying MS disease. If this was the case, the identification of the cognate antigens of our MS-mAbs would be an exceedingly difficult task and methods like those applied by Yu et al. (Yu et al. 2006) may ultimately be successful. Nevertheless, even if our MS-mAbs were the result of an initial immune response against an infectious agent and cross-

react with CNS myelin, it would be interesting to know the viral antigen but at the same time it should be possible to identify the myelin antigen.

5. Low affinity of MS-mAb: As the MS-mAbs available in our lab are the result of an antigen driven immune response and show clear signs of having undergone affinity maturation, we presume them to be of high affinity. However, they also exhibited an unexpectedly low affinity, which would make the determination of their cognate antigen extremely difficult or even impossible with the applied methods.

4.5 Relevance of self-reactive antibodies in the context of autoimmunity

We describe here auto-antibodies reacting with CNS myelin in the repertoire of CSF cePC in NB and MS patients. It is known that auto-antibodies are present in these diseases, however their relevance is a matter of debate as antibodies against myelin auto-antigens have also been described in healthy control subjects (Karni et al. 1999; Lindert et al. 1999; Mantegazza et al. 2004; Markovic et al. 2003; Reindl et al. 1999; Sun et al. 1991; Xiao et al. 1991).
However, the auto-antibodies described in this study were originally secreted by cePC and therefore the product of a truly antigen driven immune response. This indicates that reactivity to self-epitopes can be the result of a focused immune response in the CNS compartment of patients with inflammatory CNS diseases.

One of the key questions in the study of the immune response is how the normal tolerance to self-proteins is broken leading to the induction of autoimmunity. Negative selection deletes self-reactive immune cells during their development. However, the maintenance of tolerance to peripheral self-proteins or newly arising self-epitopes is not fully understood. Different processes can modify self-proteins thereby creating neo-self antigens to which the immune system has never been exposed or tolerated in the periphery.
Several post-translational modifications can create such new self-antigens and some of them have been shown to be associated with autoimmune responses (Doyle and Mamula 2001; Mantegazza et al. 2004).

Recent findings implicate apoptotic cell death in the evolution of autoimmunity (Mahoney and Rosen 2005). On the one hand, apoptotic cells play an important role in maintaining tolerance to auto-antigens and abnormalities in this process may lead to systemic autoimmunity. On the other, ‘non-default’ forms of apoptotic death can also generate new self-antigens, thus initiating immune responses.
Another explanation for the emergence of the observed auto-reactive plasma cells would be a mechanism of molecular mimicry as previously demonstrated for T cells in MS (Wucherpfennig and Strominger 1995). Yet, no known viral agent cross-reacting with the auto-specific MS-mAb could be identified. Although it is thought that in NB molecular mimicry plays a role in immune responses, no such cross-reaction could be determined in the present study.

At present, the immunopathological role of the auto-reactive antibodies described here remains cryptic. Also it is not known if these antibodies are the result of a primary or secondary effect. On the one hand, they may possess a primary demyelinating activity in the context of a CNS-directed inflammatory response. Data from animal models of MS, including rodent (Schluesener et al. 1987) and primate (Genain et al. 1995) EAE have long suggested an involvement of myelin directed antibodies in pathological processes leading to demyelination. On the other, they possibly have a scavenging activity in an environment where inflammation and myelin breakdown leaves cellular debris to be removed.

It is difficult to judge whether an infection, a neurodegenerative process or a genuine autoimmune reaction is the initial event in MS disease. The fact that binding of mAbs from cePC is largely restricted to the border of demyelinating lesions, could be interpreted as a hint for an immune response specific for novel epitopes evolving during tissue destruction, rather than a primary demyelinating one. However, the elucidation of the antigen specificity would be indispensable information to judge the relevance of these antibodies.

5 MATERIAL AND METHODS

5.1 Standard buffers

PBS	150mM	NaCl
	30mM	KCl
	1.5mM	KH_2PO_4
	6mM	Na_2HPO_4

TBS 10mM Tris/HCL pH: 8.0
150mM NaCl

LB 1 % (w/v) Bacto Trypton
0.5 % (w/v) Hefeextrakt
0.5 % (w/v) NaCl

5.2 Patients, cerebrospinal fluid and plasma cell sorting

Upon receiving informed consent from each patient, we obtained 7-10 ml of CSF during a spinal tap intended for diagnostic purposes. The study was approved by the local ethics committee. CSF cells were incubated with FITC labeled anti-human CD138 monoclonal antibody (Serotec) at 4 °C for 20 minutes. Single CD138+ plasma cells were sorted on a FACS-Aria (Becton Dickinson) into individual wells of 96-well PCR plates, each containing 20µl of reverse-transcription reaction buffer and stored at -80 °C until further processing.

5.3 Single cell-RT-PCR and antibody cloning

For initial single cell RT-PCR, reverse primers specific for the constant regions of Ig genes (IgG-heavy chain, λL chain, κL chain) and forward primers specific for the conserved framework region 1 complementary for H or L chain Ig variable region families were used, as described (Andris-Widhopf et al. 2001; Owens et al. 2003).

PCR products were resolved on a 1.2% agarose gel. Appropriately sized PCR products were extracted from agarose gels and sent for automated sequencing (Synergene Biotech). Ig sequences from cePCs were identified by assembling groups of identical Ig H chain V region genes (SeqMan, DNASTAR) and were further analyzed for germline segment usage and H-CDR3 mutations via the V-Quest tool on the IMGT website (Giudicelli et al. 2004).

H and L chain V region genes of the MOG-specific antibody 8-18c5 were amplified from 8-18c5 hybridoma cells (kindly provided by C. Linington, University of Aberdeen, Scottland) mRNA by RT-PCR using mouse Ig specific primers (Breithaupt et al. 2003) and cloned into the expression vectors described later on to obtain chimeric anti-MOG antibodies containing the murine antigen binding region of 8-18c5 and a human IgG1 Fc-portion (hu8-18c5).

To avoid PCR cross-contamination between different patients' repertoires and to rule out contamination of PCR components, we took a number of precautions: 1. A number of negative controls, e.g. wells of PCR plates in which no plasma cells had been deposited, were run on each PCR plate; 2. The areas in which PCR setup, PCR analysis by gel electrophoresis and cloning of PCR products were performed, are designated spaces, which are physically separated and equipped with designated sets of pipetters; 3. Fresh aliquots of PCR components were used for each patient's CSF plasma cell repertoire.

Ig H and L chain PCR products from cePC were cloned into vectors containing the constant region of IgG1, Igκ or Igλ (kindly provided by H. Wardemann, Max-Planck Institut für Infektionsbiologie, Berlin, Germany) as described (Wardemann et al. 2003). Briefly, for cloning IgH and Igλ genes, restriction sites were introduced by the 2nd PCR. Digested IgH and Igλ PCR products were gel-purified and directly cloned into expression vectors containing human IgG1 or Igλ constant regions. Igκ 2nd-PCR reactions were performed with a consensus κ primer and did not allow direct cloning. Instead, after sequencing, 1 µl of the purified 1st round RT-PCR product was used for a 2nd round of PCR with restriction-site containing specific κ-gene primers. After purification, digestion and gel-purification, Igκ PCR products were cloned into the Igκ expression vector containing the Igκ constant regions. Plasmids were sequenced to select clones with inserts identical to the original PCR product.

5.4 Antibody production and purification

293-T human embryonic kidney fibroblasts (ATCC) were cultured in D-MEM supplemented with ultra-low IgG FCS, penicillin-streptomycin and L-glutamin (all from Invitrogen). Co-transfection of 293-T cells with Ig H and L chain encoding plasmid DNA was performed by calcium phosphate precipitation (Chen and Okayama 1988). Thereafter the cells were cultured in serum free D-MEM supplemented with 1% Nutridoma SP (Roche). Supernatants were collected after 8 days of culture and recombinant mAbs (IgG1) were purified by affinity chromatography over protein G columns (Amersham Biosciences). Murine hybridoma 8-18c5 (m8-18c5) was a kind gift of C. Linington, University of Aberdeen, Scottland. Where desired, antibodies were biotinylated using NHS-biotin (Sigma) following the manufacturer's instructions.

5.5 Western analysis of high-density protein macroarrays

A hEx1 protein array comprising expression products of 37.000 independent human fetal brain cDNA clones was obtained from the Deutsches Ressourcenzentrum für Genomforschung GmbH (RZPD) (http://www.rzpd.de) and probed with mAb according to the manufacturer's recommendations. Briefly, macroarrays were rinsed in 96% Ethanol at RT and subsequently washed with water following by TBS, 0.005% Tween20, 0.5% Triton X-100 and lastly with TBS. Macroarrays were then blocked for 2h at RT with 1% milk TBS, 0.5% Tween20. mAbs were added to the blocking solution at 10ug/ml and the arrays were incubated o.n. at 4°C. After washing with TBS, 0.5% Tween20, anti human IgG-AP (1:5'000, SIGMA) in blocking solution was added and incubated at RT for 2h. After washing, positive spots were visualized using ECF substrate (Amersham Biosciences) and subsequently scanned on FluorImager595 (Molecular Dynamics).

5.6 Expression and purification of His-tag proteins from RZPD

Proteins were purified under denaturating conditions using standard protocols (described in detail in Ni-NTA Spin Handbook from QIAGEN). Briefly, rzpd-clones were cultured in LB medium containing 100ug/ml ampicilin and 25ug/ml kanamycin at 37°C with vigorous shaking until the OD_{600} reached 0.6. Then, IPTG was added to a

final concentration of 1mM and the culture was grown for further 4 hours. Cells were harvested by centrifugation and resuspended in 8M urea, 0.1M NaH_2PO_4, 0.01M Tris-Cl pH 8 (buffer B). They were then incubated for 1 hour at RT with agitation and subsequently, the lysed cells were centrifugated (30 min, RT, 10'000g) to pellet the cellular debris. After equilibration of Ni-NTA spin column with buffer B, the cleared lysate supernatant was added. Columns were washed with 8M urea, 0.1M NaH_2PO_4, 0.01M Tris-Cl pH 6.3 and subsequently, proteins were eluted with 8M urea, 0.1M NaH_2PO_4, 0.01M Tris-Cl pH 4.5.

5.7 ELISA

Wells of polycarbonate plates (MaxiSorp, Nunc) were coated with respective protein o.n. at 4°C. Wells were then blocked with PBS containing 0.05% Tween-20 (PBS-T) and 3% BSA (SIGMA). Antibodies were added at 10ug/ml in blocking buffer and incubated for 1 hour at 37 °C. Bound antibody was detected with a HRP labeled secondary antibody, revealed by tetramethylbenzidine (Pierce) and analyzed at 450nm using a microplate reader (Bio-Rad).

Coated proteins

Borrelia-ELISA: 5ug/well Bb protein lysate (kindly provided by Dr. O. Péter, Division de Médecine Préventive Hospitalière, Lausanne, Switzerland.)

p41-ELISA: 0.25ug/well purified recombinant *Borrelia afzelii* p41 antigen (PKo) (Mikrogen)

E.coli-ELISA: 5ug/well E.coli lysate

MOG-ELISA: 1ug/well of a recombinant form of the extracellular domain of human MOG (rMOG, kind gift of M. Reindl, Innsbruck Medical University, Austria)

MBP-ELISA: 1ug/well of purified human MBP

RZPD-protein ELISA: 1ug/well purified RZPD-protein

Primary antibodies

Recombinant human monoclonal antibodies: 10ug/ml

Chimeric anti-MOG hu8-18c5-antibody: 10ug/ml

Rat anti-MBP antibody (Serotec): 10ug/ml

Secondary antibodies

HRP labeled goat anti-human IgG+IgM (1:100'000, Jackson ImmunoResearch)

HRP labeled goat anti-rat IgG antibody (1:50'000 Jackson ImmunoResearch)

Streptavidin-HRP conjugate (1:500, SIGMA)

5.8 Competition Assays

Initially, saturation experiments were conducted to obtain the half-maximal binding capacity of biotinylated RA77 for 5ug Bb lysate and 0.25 ug recombinant p41, as well as of biotinylated 8-18c5. ELISA plates were coated with 5ug/well Bb lysate, 0.25ug/well recombinant p41 or 1ug/well rMOG and incubated with increasing concentrations of biotinylated RA77 or both hybridoma derived (m8-18c5) and recombinant chimeric 8-18c5 (hu8-18c5), respectively. Bound biotinylated antibodies were detected with streptavidin-HRP and TMB as described above. Competition experiments of biotinylated versus non-biotinylated antibodies were conducted to obtain the affinity of RA77 for Bb lysate or recombinant p41, and m8-18c5 or hu8-18c5 for rMOG. Increasing concentrations of non-biotinylated antibodies were added to ELISA wells coated with the respective antigens in the presence of the previously determined 50% saturation concentration of the corresponding biotinylated antibody. After 1 hour at 37 °C, wells were washed and bound biotinylated antibodies detected by streptavidin-HRP and TMB. The affinity (Ki) was calculated according to Cheng and Prusoff (Cheng and Prusoff 1973).

5.9 Immunoprecipitation

Protein G sepharose (Amersham) was incubated with RA77 or human IgG1 in PBS for 3 hours at 4 °C. The sepharose-antibody complex was washed once and resuspended in IP-buffer (20mM HEPES, 5mM MgCl, 0.05% NP40, 120mM NaCl, and protease inhibitors (Roche)) and Bb lysate was added. Reactions were incubated overnight at 4 °C, centrifuged at 500g and resuspended in IP-buffer. The resuspended sepharose was added onto pre-equilibrated empty columns (Amersham) and washed with IP buffer. Immunoprecipitated proteins were eluted with 0.1M glycine buffer pH 3 and separated by reducing SDS/PAGE. Proteins were visualized by silver staining (Pierce). Bands for MALDI-TOF analysis were excised from duplicate gels stained with Coomassie Blue (Pierce).

5.10 MALDI-TOF Analysis

MALDI-TOF analyses were performed by Dr. S. Chesnov and Dr. P. Hunziker (University of Zurich, Functional Genomics Center Zurich). Gel bands were cut in small pieces. In-gel digestion was performed according to Schrimpf et al. (Schrimpf et al. 2001) by re-hydrating the washed and dried gel pieces with 20 µl trypsin solution (modified trypsin, Promega). After digestion over night at 37°C the supernatant was removed and the gel pieces were extracted with 20µl 5% (v/v) formic acid. 1µl of the combined supernatants was mixed with 1µl of a saturated solution of alpha-cyano-4-hydroxycinnamic acid (Fluka) in 0.1% (v/v) trifluoroacetic acid/acetonitrile (2:1) and 1 µl was applied onto the MALDI target. Mass spectra were recorded on a Biflex III (Bruker Daltonics). The resulting list of peptide masses was searched against MSDB protein database using Mascot (http://www.matrixscience.com).

5.11 Immunofluorescence stainings

Bb and Tp spirochetes attached to microscopy slides (Euroimmun) were stained with RA77-Biotin and either Bb specific anti-p41 antibody (mAb 1 C11, kindly provided by Dr. Fingerle, Munich) or anti-Tp positive human control serum (Euroimmun), respectively. Immunofluorescence stainings of frozen human tissue were performed with biotinylated RA17, RA20, RA77 and human IgG (Fitzgerald) on sections of post-mortem white matter, facial nerve, colon, lung, and kidney tissue (kindly provided by the NICHD Brain and Tissue bank for Developmental Disorders under contracts N01-HD-4-3368 and N01-HD-4-3383) with 10µg/ml per mAb. Sections were fixed with formaldehyde, permeabilized with Triton X-100, treated with an avidin-biotin blocking protocol (Vectorlabs) and blocked with 10% normal goat serum in PBS supplemented with 0.2% Triton X-100. Subsequent incubation with individual biotinylated mAbs took place overnight at 4°C, if designated, along with antibodies for simultaneous staining of CNS resident cell types (mouse anti-neurofilament200 (SIGMA), neurons; mouse anti-MOG 8-18c5-Ab, myelin). After washes in PBS, bound biotinylated mAbs were detected with streptavidin-ALEXA546, streptavidin-ALEXA488, anti-mouse IgG-ALEXA488, anti-mouse IgG-ALEXA546 or anti-humanIgG-ALEXA546 conjugates (1:700, Molecular Probes) as secondary antibodies. Nuclei were counterstained with Hoechst 55432 and tissue sections mounted with an aqueous mounting medium.

5.12 Isolation of human myelin

Human CNS white matter tissue was obtained at autopsy from individuals without a history of neurological disease (A00-329 from N. Schaeren-Wiemers) or MS (1709 kindly provided by the NICHD Brain and Tissue bank for Developmental Disorders under contracts N01-HD-4-3368 and N01-HD-4-3383).
Myelin was isolated as described (Norton and Poduslo 1973), with adaptations. Briefly, CNS white matter was homogenized in 0.25M sucrose in 10mM Hepes, 2mM EGTA pH 7.4. After centrifugation, 2M sucrose prepared in 10mM Hepes, 2mM EGTA pH 7.4 was added to the supernatant to finally yield a 1.4M sucrose suspension. A gradient was set up as following: 0.7ml of 0.25M sucrose, 2ml of 0.85M sucrose, 9ml homogenate suspension, 1ml of 2M sucrose. Ultracentrifugation was performed for 20 hours at 4°C at 25'000rpm. The white cloudy layer obtained on the top, containing the compact myelin, was again homogenized in 10mM Hepes, 2mM EGTA and centrifuged at 25'000rpm for 2 hours at 4°C. After removal of the lipid rich layer from the top, supernatant was decanted and the pellet resolved in sterile water. Myelin was collected and stored at − 80 °C until further use. The protein concentration was calculated using a BSA standard curve by Bradford's method (Pierce).

5.13 Myelin western blot

20ug myelin was loaded on a gradient acryl amid gel and run over night at 18V. Transfer onto Immuno-Blot PVDF 0,2um membrane (Biorad) was performed at 50V in a 192mM Glycine, 25mM Tris buffer. Membranes were blocked for 2 hours at RT with 3% TOP-Block (Fluka) in TBS, 0.05% Tween20. mAbs, anti-MAG antibody (produced by Anthony Heape, Finland) and anti-CNPase antibody (Covance) were incubated at a concentration of 10ug/ml in 1% TOP-Block, TBS, 0.05% Tween20 over night at 4°C. Secondary anti-human IgG conjugated to IRDye®700DX (Rockland Immunochemicals), anti-mouse IgG-ALEXA680 (Molecular Probes) and anti-rabbit IgG IRDye®800DX (Rockland Immunochemicals) were diluted 1:2'500 in 1% TOP-Block, TBS, 0.05% Tween20 and fluorescence signals were measured using the Odyssey® Infrared Imaging System (LI-COR Bioscience).

5.14 FACS analysis of myelin vesicles

FACS experiments with human myelin was performed as described (Breij et al. 2006). Briefly, human myelin (15ug) and 1ug of biotinylated mAb or hu8-18c5 were added to 100ul PBS pH 7.4 in a 96-well V-bottom plate and incubated overnight at 4°C. Unbound antibodies were removed by washing the myelin in PBS (4500rpm, 4min, 3x). Myelin was subsequently incubated with Streptavidin-APC (1:10'000, BD Bioscience) for 30 min at 4°C. After washing in PBS (4500rpm, 4 min, 3x) the myelin was diluted in PBS, 0.5%BSA and analyzed using a FACS Calibur (BD Bioscience).

5.15 FACS analysis of MOG transfected Jurkat cells

Jurkat cells were stably transfected using Lipofectamine (Invitrogen) to express human MOG on their surface (plasmid kind gift of M. Reindl, Innsbruck Medical University, Austria). For FACS analysis, MOG-transfected Jurkat cells were incubated with 10µg/ml of each MS-mAb, chimeric anti-MOG hu8-18c5-antibody or human IgG1 isotype control for 10 min at 4°C. After a wash, bound antibodies were detected by addition of anti-human IgG-APC conjugate (BD Biosciences) scanning in a FACS Calibur (BD Biosciences).

6 REFERENCES

1. Alaedini, A. and N. Latov. 2005 "Antibodies against OspA epitopes of Borrelia burgdorferi cross-react with neural tissue." *J.Neuroimmunol.* 159(1-2): 192-195.

2. Alitalo, A. et al. 2002 "Complement inhibitor factor H binding to Lyme disease spirochetes is mediated by inducible expression of multiple plasmid-encoded outer surface protein E paralogs." *J.Immunol.* 169(7): 3847-3853.

3. Alitalo, A. et al. 2001 "Complement evasion by Borrelia burgdorferi: serum-resistant strains promote C3b inactivation." *Infect.Immun.* 69(6): 3685-3691.

4. Aloisi, F. and R. Pujol-Borrell. 2006 "Lymphoid neogenesis in chronic inflammatory diseases." *Nat.Rev.Immunol.* 6(3): 205-217.

5. Andris-Widhopf, J. et al. 2001. "Generation of Antibody Libraries: PCR Amplification and Assembly of Light- and Heavy-chain Coding Sequences." Pp. 1-113 in *Phage Display A Laboratory Manual.* Edited by Carlos F.Barbas III et al.CSHL Press.

6. Baranzini, S. E. et al. 1999 "B cell repertoire diversity and clonal expansion in multiple sclerosis brain lesions." *J.Immunol.* 163(9): 5133-5144.

7. Baranzini, S. E. et al. 2002 "Analysis of antibody gene rearrangement, usage, and specificity in chronic focal encephalitis." *Neurology* 58(5): 709-716.

8. Barbour, A. G. and S. F. Hayes. 1986 "Biology of Borrelia species." *Microbiol.Rev.* 50(4): 381-400.

9. Barthold, S. W. and L. K. Bockenstedt. 1993 "Passive immunizing activity of sera from mice infected with Borrelia burgdorferi." *Infect.Immun.* 61(11): 4696-4702.

10. Benach, J. L. et al. 1983 "Spirochetes isolated from the blood of two patients with Lyme disease." *N.Engl.J.Med.* 308(13): 740-742.

11. Berger, T. et al. 2003 "Antimyelin antibodies as a predictor of clinically definite multiple sclerosis after a first demyelinating event." *N.Engl.J.Med.* 349(2): 139-145.

12. Brahamsha, B. and E. P. Greenberg. 1988 "Biochemical and cytological analysis of the complex periplasmic flagella from Spirochaeta aurantia." *J.Bacteriol.* 170(9): 4023-4032.

13. Breij, E. C. et al. 2006 "Myelin flow cytometry assay detects enhanced levels of antibodies to human whole myelin in a subpopulation of multiple sclerosis patients." *J.Neuroimmunol.* 176(1-2): 106-114.

14. Breithaupt, C. et al. 2003 "Structural insights into the antigenicity of myelin oligodendrocyte glycoprotein." *Proc.Natl.Acad.Sci.U.S.A* 100(16): 9446-9451.

15. Burgdorfer, W. et al. 1982 "Lyme-Disease - A Tick-Borne Spirochetosis." *Science* 216(4552): 1317-1319.

16. Burgoon, M. P. et al. 2006 "Recombinant antibodies generated from both clonal and less abundant plasma cell immunoglobulin G sequences in subacute sclerosing panencephalitis brain are directed against measles virus." *J.Neurovirol.* 12(5): 398-402.

17. Burgoon, M. P. et al. 2005 "Laser-capture microdissection of plasma cells from subacute sclerosing panencephalitis brain reveals intrathecal disease-relevant antibodies." *Proc.Natl.Acad.Sci.U.S.A* 102(20): 7245-7250.

18. Burgoon, M. P. et al. 1999 "Cloning the antibody response in humans with inflammatory central nervous system disease: analysis of the expressed IgG repertoire in subacute sclerosing panencephalitis brain reveals disease-relevant antibodies that recognize specific measles virus antigens." *J.Immunol.* 163(6): 3496-3502.

19. Cannella, B. and C. S. Raine. 1995 "The adhesion molecule and cytokine profile of multiple sclerosis lesions." *Ann.Neurol.* 37(4): 424-435.

20. Casjens, S. et al. 2000 "A bacterial genome in flux: the twelve linear and nine circular extrachromosomal DNAs in an infectious isolate of the Lyme disease spirochete Borrelia burgdorferi." *Mol.Microbiol.* 35(3): 490-516.

21. Cepok, S. et al. 2005 "Identification of Epstein-Barr virus proteins as putative targets of the immune response in multiple sclerosis." *J.Clin.Invest* 115(5): 1352-1360.

22. Cepok, S. et al. 2003 "The immune response at onset and during recovery from Borrelia burgdorferi Meningoradiculitis." *Archives of Neurology* 60(6): 849-855.

23. Chen, C. A. and H. Okayama. 1988 "Calcium phosphate-mediated gene transfer: a highly efficient transfection system for stably transforming cells with plasmid DNA." *Biotechniques* 6(7): 632-638.

24. Cheng, Y. and W. H. Prusoff. 1973 "Relationship Between Inhibition Constant (K1) and Concentration of Inhibitor Which Causes 50 Per Cent Inhibition (I50) of An Enzymatic-Reaction." *Biochemical Pharmacology* 22(23): 3099-3108.

25. Clark, J. R. et al. 1985 "Facial paralysis in Lyme disease." *Laryngoscope* 95(11): 1341-1345.

26. Colombo, M. et al. 2000 "Accumulation of clonally related B lymphocytes in the cerebrospinal fluid of multiple sclerosis patients." *J.Immunol.* 164(5): 2782-2789.

27. Connolly, S. E. and J. L. Benach. 2005 "The versatile roles of antibodies in Borrelia infections." *Nat.Rev.Microbiol.* 3(5): 411-420.

28. Corcione, A. et al. 2004 "Recapitulation of B cell differentiation in the central nervous system of patients with multiple sclerosis." *Proc.Natl.Acad.Sci.U.S.A* 101(30): 11064-11069.

29. Dai, Z. et al. 1993 "Molecular mimicry in Lyme disease: monoclonal antibody H9724 to B. burgdorferi flagellin specifically detects chaperonin-HSP60." *Biochim.Biophys.Acta* 1181(1): 97-100.

30. de Wildt, R. M. et al. 1997 "A new method for the analysis and production of monoclonal antibody fragments originating from single human B cells." *J.Immunol.Methods* 207(1): 61-67.

31. Doyle, H. A. and M. J. Mamula. 2001 "Post-translational protein modifications in antigen recognition and autoimmunity." *Trends Immunol.* 22(8): 443-449.

32. Fikrig, E. et al. 1994 "Sera from patients with chronic Lyme disease protect mice from Lyme borreliosis." *J.Infect.Dis.* 169(3): 568-574.

33. Franciotta, D. et al. 2005 "Analysis of Chlamydia pneumoniae-specific oligoclonal bands in multiple sclerosis and other neurologic diseases." *Acta Neurol.Scand.* 112(4): 238-241.

34. Fraser, C. M. et al. 1997 "Genomic sequence of a Lyme disease spirochaete, Borrelia burgdorferi." *Nature* 390(6660): 580-586.

35. Fujinami, R. S. et al. 2006 "Molecular mimicry, bystander activation, or viral persistence: infections and autoimmune disease." *Clin.Microbiol.Rev.* 19(1): 80-94.

36. Genain, C. P. et al. 1999 "Identification of autoantibodies associated with myelin damage in multiple sclerosis." *Nat.Med.* 5(2): 170-175.

37. Genain, C. P. et al. 1995 "Antibody facilitation of multiple sclerosis-like lesions in a nonhuman primate." *J.Clin.Invest* 96(6): 2966-2974.

38. Gilden, D. H. et al. 1996 "The search for virus in multiple sclerosis brain." *Mult.Scler.* 2(4): 179-183.

39. Giudicelli, V., D. Chaume, and M. P. Lefranc. 2004 "IMGT/V-QUEST, an integrated software program for immunoglobulin and T cell receptor V-J and V-D-J rearrangement analysis." *Nucleic Acids Res.* 32(Web Server issue): W435-W440.

40. Gross, D. M. et al. 1998 "Identification of LFA-1 as a candidate autoantigen in treatment-resistant Lyme arthritis." *Science* 281(5377): 703-706.

41. Hansen, K., M. Cruz, and H. Link. 1990 "Oligoclonal Borrelia burgdorferi-specific IgG antibodies in cerebrospinal fluid in Lyme neuroborreliosis." *J.Infect.Dis.* 161(6): 1194-1202.

42. Haubold, K. et al. 2004 "B-lymphocyte and plasma cell clonal expansion in monosymptomatic optic neuritis cerebrospinal fluid." *Annals of Neurology* 56(1): 97-107.

43. Hemmer, B. et al. 1999 "Identification of candidate T-cell epitopes and molecular mimics in chronic Lyme disease." *Nat.Med.* 5(12): 1375-1382.

44. Hengge, U. R. et al. 2003 "Lyme borreliosis." *Lancet Infect.Dis.* 3(8): 489-500.

45. Jacobsen, M. et al. 2003 "Clonal accumulation of activated CD8+ T cells in the central nervous system during the early phase of neuroborreliosis." *J.Infect.Dis.* 187(6): 963-973.

46. Kaiser, R. 1995 "Intrathecal immune response in patients with neuroborreliosis: specificity of antibodies for neuronal proteins." *J.Neurol.* 242(5): 319-325.

47. Kaiser, R. 1998 "Neuroborreliosis." *J.Neurol.* 245(5): 247-255.

48. Kalish, R. A. et al. 2001 "Persistence of immunoglobulin M or immunoglobulin G antibody responses to Borrelia burgdorferi 10-20 years after active Lyme disease." *Clin.Infect.Dis.* 33(6): 780-785.

49. Kanter, J. L. et al. 2006 "Lipid microarrays identify key mediators of autoimmune brain inflammation." *Nat.Med.* 12(1): 138-143.

50. Karni, A. et al. 1999 "Elevated levels of antibody to myelin oligodendrocyte glycoprotein is not specific for patients with multiple sclerosis." *Arch.Neurol.* 56(3): 311-315.

51. Kenealy, S. J., M. A. Pericak-Vance, and J. L. Haines. 2003 "The genetic epidemiology of multiple sclerosis." *J.Neuroimmunol.* 143(1-2): 7-12.

52. Kim, J. K. et al. 2003 "Multiple sclerosis: an important role for post-translational modifications of myelin basic protein in pathogenesis." *Mol.Cell Proteomics.* 2(7): 453-462.

53. Kimata, H. et al. 1992 "Interleukin 8 (IL-8) selectively inhibits immunoglobulin E production induced by IL-4 in human B cells." *J.Exp.Med.* 176(4): 1227-1231.

54. Kochi, S. K., R. C. Johnson, and A. P. Dalmasso. 1991 "Complement-mediated killing of the Lyme disease spirochete Borrelia burgdorferi. Role of antibody in formation of an effective membrane attack complex." *J.Immunol.* 146(11): 3964-3970.

55. Kohler, G. and C. Milstein. 1975 "Continuous cultures of fused cells secreting antibody of predefined specificity." *Nature* 256(5517): 495-497.

56. Kraiczy, P. et al. 2003 "Immune evasion of Borrelia burgdorferi: mapping of a complement-inhibitor factor H-binding site of BbCRASP-3, a novel member of the Erp protein family." *Eur.J.Immunol.* 33(3): 697-707.

57. Kruger, H., D. Englert, and K. W. Pflughaupt. 1981 "Demonstration of oligoclonal immunoglobulin G in Guillain-Barre syndrome and lymphocytic meningoradiculitis by isoelectric focusing." *J.Neurol.* 226(1): 15-24.

58. Krumbholz, M. et al. 2006 "Chemokines in multiple sclerosis: CXCL12 and CXCL13 up-regulation is differentially linked to CNS immune cell recruitment." *Brain* 129(Pt 1): 200-211.

59. Krumbholz, M. et al. 2005 "BAFF is produced by astrocytes and up-regulated in multiple sclerosis lesions and primary central nervous system lymphoma." *J.Exp.Med.* 201(2): 195-200.

60. Kuhle, J. et al. 2007 "Lack of association between antimyelin antibodies and progression to multiple sclerosis." *N.Engl.J.Med.* 356(4): 371-378.

61. Lane, R. S., J. Piesman, and W. Burgdorfer. 1991 "Lyme borreliosis: relation of its causative agent to its vectors and hosts in North America and Europe." *Annu.Rev.Entomol.* 36: 587-609.

62. Li, Z. et al. 1993 "A species-specific periplasmic flagellar protein of Serpulina (Treponema) hyodysenteriae." *J.Bacteriol.* 175(24): 8000-8007.

63. Lindert, R. B. et al. 1999 "Multiple sclerosis: B- and T-cell responses to the extracellular domain of the myelin oligodendrocyte glycoprotein." *Brain* 122 (Pt 11): 2089-2100.

64. Linington, C. et al. 1988 "Augmentation of demyelination in rat acute allergic encephalomyelitis by circulating mouse monoclonal antibodies directed against a myelin/oligodendrocyte glycoprotein." *Am.J.Pathol.* 130(3): 443-454.

65. Link, H. 1978 "Immunoglobulins in multiple sclerosis." *Adv.Exp.Med.Biol.* 100: 639-640.

66. Logigian, E. L., R. F. Kaplan, and A. C. Steere. 1990 "Chronic neurologic manifestations of Lyme disease." *N.Engl.J.Med.* 323(21): 1438-1444.

67. Lolli, F. et al. 2005 "An N-glucosylated peptide detecting disease-specific autoantibodies, biomarkers of multiple sclerosis." *Proc.Natl.Acad.Sci.U.S.A* 102(29): 10273-10278.

68. Louis Reik, Jr. 1991. *Lyme Disease and the Nervous System.*Thieme Medical Publishers.

69. Love, S. 2006 "Demyelinating diseases." *J.Clin.Pathol.* 59(11): 1151-1159.

70. Lucchinetti, C. et al. 2000 "Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination." *Ann.Neurol.* 47(6): 707-717.

71. Lunemann, J. D. et al. 2006 "CSF-Infiltrating CD4+ T Cells Recognize Borrelia burgdorferi Lysine-Enriched Protein Domains And CNS Auto-Antigens In Early Lyme Encephalitis." *Infect.Immun.*

72. Mahoney, J. A. and A. Rosen. 2005 "Apoptosis and autoimmunity." *Curr.Opin.Immunol.* 17(6): 583-588.

73. Mantegazza, R. et al. 2004 "Anti-MOG autoantibodies in Italian multiple sclerosis patients: specificity, sensitivity and clinical association." *Int.Immunol.* 16(4): 559-565.

74. Manz, R. A. et al. 2005 "Maintenance of serum antibody levels." *Annu.Rev.Immunol.* 23: 367-386.

75. Markovic, M. et al. 2003 "Antibodies against myelin oligodendrocyte glycoprotein in the cerebrospinal fluid of multiple sclerosis patients." *J.Neurol.Sci.* 211(1-2): 67-73.

76. Marta, C. B. et al. 2005 "Pathogenic myelin oligodendrocyte glycoprotein antibodies recognize glycosylated epitopes and perturb oligodendrocyte physiology." *Proc.Natl.Acad.Sci.U.S.A* 102(39): 13992-13997.

77. Martin, Mdel P. and N. L. Monson. 2007 "Potential role of humoral immunity in the pathogenesis of multiple sclerosis (MS) and experimental autoimmune encephalomyelitis (EAE)." *Front Biosci.* 12: 2735-2749.

78. Martin, R. et al. 1988a "Persistent intrathecal secretion of oligoclonal, Borrelia burgdorferi-specific IgG in chronic meningoradiculomyelitis." *J.Neurol.* 235(4): 229-233.

79. Martin, R. et al. 1988b "Borrelia burgdorferi--specific and autoreactive T-cell lines from cerebrospinal fluid in Lyme radiculomyelitis." *Ann.Neurol.* 24(4): 509-516.

80. Menge, T. et al. 2005 "Antibody responses against galactocerebroside are potential stage-specific biomarkers in multiple sclerosis." *J.Allergy Clin.Immunol.* 116(2): 453-459.

81. Migliorini, P. et al. 2005 "The immune response to citrullinated antigens in autoimmune diseases." *Autoimmun.Rev.* 4(8): 561-564.

82. Montgomery, R. R., M. H. Nathanson, and S. E. Malawista. 1994 "Fc- and non-Fc-mediated phagocytosis of Borrelia burgdorferi by macrophages." *J.Infect.Dis.* 170(4): 890-893.

83. Moore, K. W. et al. 2001 "Interleukin-10 and the interleukin-10 receptor." *Annu.Rev.Immunol.* 19: 683-765.

84. Moscarello, M. A., F. G. Mastronardi, and D. D. Wood. 2007 "The role of citrullinated proteins suggests a novel mechanism in the pathogenesis of multiple sclerosis." *Neurochem.Res.* 32(2): 251-256.

85. Moscarello, M. A. et al. 1994 "Myelin in multiple sclerosis is developmentally immature." *J.Clin.Invest* 94(1): 146-154.

86. Nagi, K. S., R. Joshi, and R. K. Thakur. 1996 "Cardiac manifestations of Lyme disease: a review." *Can.J.Cardiol.* 12(5): 503-506.

87. Norton, W. T. and S. E. Poduslo. 1973 "Myelination in rat brain: method of myelin isolation." *J.Neurochem.* 21(4): 749-757.

88. Noseworthy, J. H. et al. 2000 "Multiple sclerosis." *N.Engl.J.Med.* 343(13): 938-952.

89. Oschmann, P. et al. 1998 "Stages and syndromes of neuroborreliosis." *J.Neurol.* 245(5): 262-272.

90. Owens, G. et al. 2004 "A comparative single-cell analysis of the CD19+ and CD138+ cell antibody repertoires in the cerebrospinal fluid of patients with multiple sclerosis." *Journal of Neuroimmunology* 154(1-2): 93.

91. Owens, G. P. et al. 1998 "Restricted use of VH4 germline segments in an acute multiple sclerosis brain." *Ann.Neurol.* 43(2): 236-243.

92. Owens, G. P. et al. 2003 "Single-cell repertoire analysis demonstrates that clonal expansion is a prominent feature of the B cell response in multiple sclerosis cerebrospinal fluid." *Journal of Immunology* 171(5): 2725-2733.

93. Paul, H., H. J. Gerth, and R. Ackermann. 1987 "Infectiousness for humans of Ixodes ricinus containing Borrelia burgdorferi." *Zentralbl.Bakteriol.Mikrobiol.Hyg.[A]* 263(3): 473-476.

94. Peterson, P. K. et al. 1984 "Human phagocyte interactions with the Lyme disease spirochete." *Infect.Immun.* 46(2): 608-611.

95. Pfister, H. W., B. Wilske, and K. Weber. 1994 "Lyme borreliosis: basic science and clinical aspects." *Lancet* 343(8904): 1013-1016.

96. Piddlesden, S. J. et al. 1993 "The demyelinating potential of antibodies to myelin oligodendrocyte glycoprotein is related to their ability to fix complement." *Am.J.Pathol.* 143(2): 555-564.

97. Polman, C. H. et al. 2005 "Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria"." *Ann.Neurol.* 58(6): 840-846.

98. Qin, Y. et al. 1998 "Clonal expansion and somatic hypermutation of V(H) genes of B cells from cerebrospinal fluid in multiple sclerosis." *J.Clin.Invest* 102(5): 1045-1050.

99. Ransohoff, R. M., P. Kivisakk, and G. Kidd. 2003 "Three or more routes for leukocyte migration into the central nervous system." *Nat.Rev.Immunol.* 3(7): 569-581.

100. Reindl, M. et al. 1999 "Antibodies against the myelin oligodendrocyte glycoprotein and the myelin basic protein in multiple sclerosis and other neurological diseases: a comparative study." *Brain* 122 (Pt 11): 2047-2056.

101. Richard A.Goldsby, Thomas J.Kindt, and Barbara A.Osborne. 2000. *Kuby Immunology.* 4th ed.Freeman and Company.

102. Ritchie, A. M. et al. 2004 "Comparative analysis of the CD19+ and CD138+ cell antibody repertoires in the cerebrospinal fluid of patients with multiple sclerosis." *J.Immunol.* 173(1): 649-656.

103. Rupprecht, T. A. et al. 2005a "CXCL11 is involved in leucocyte recruitment to the central nervous system in neuroborreliosis." *J.Neurol.* 252(7): 820-823.

104. Rupprecht, T. A. et al. 2005b "The chemokine CXCL13 (BLC): a putative diagnostic marker for neuroborreliosis." *Neurology* 65(3): 448-450.

105. Schluesener, H. J. et al. 1987 "A monoclonal antibody against a myelin oligodendrocyte glycoprotein induces relapses and demyelination in central nervous system autoimmune disease." *J.Immunol.* 139(12): 4016-4021.

106. Schneider, P. 2005 "The role of APRIL and BAFF in lymphocyte activation." *Curr.Opin.Immunol.* 17(3): 282-289.

107. Schrimpf, S. P. et al. 2001 "A two-dimensional protein map of Caenorhabditis elegans." *Electrophoresis* 22(6): 1224-1232.

108. Schwarz, M. et al. 2006 "Serum anti-Glc(alpha1,4)Glc(alpha) antibodies as a biomarker for relapsing-remitting multiple sclerosis." *J.Neurol.Sci.* 244(1-2): 59-68.

109. Serafini, B. et al. 2004 "Detection of ectopic B-cell follicles with germinal centers in the meninges of patients with secondary progressive multiple sclerosis." *Brain Pathol.* 14(2): 164-174.

110. Shapiro-Shelef, M. and K. Calame. 2005 "Regulation of plasma-cell development." *Nat.Rev.Immunol.* 5(3): 230-242.

111. Sigal, L. H. and S. Williams. 1997 "A monoclonal antibody to Borrelia burgdorferi flagellin modifies neuroblastoma cell neuritogenesis in vitro: a possible role for autoimmunity in the neuropathy of Lyme disease." *Infect.Immun.* 65(5): 1722-1728.

112. Sindern, E. and J. P. Malin. 1995 "Phenotypic analysis of cerebrospinal fluid cells over the course of Lyme meningoradiculitis." *Acta Cytol.* 39(1): 73-75.

113. Smith-Jensen, T. et al. 2000 "Comparison of immunoglobulin G heavy-chain sequences in MS and SSPE brains reveals an antigen-driven response." *Neurology* 54(6): 1227-1232.

114. Steere, A. C., R. T. Schoen, and E. Taylor. 1987 "The clinical evolution of Lyme arthritis." *Ann.Intern.Med.* 107(5): 725-731.

115. Storch, M. K. et al. 1998 "Multiple sclerosis: in situ evidence for antibody- and complement-mediated demyelination." *Ann.Neurol.* 43(4): 465-471.

116. Sun, J. et al. 1991 "T and B cell responses to myelin-oligodendrocyte glycoprotein in multiple sclerosis." *J.Immunol.* 146(5): 1490-1495.

117. Taga, T. and T. Kishimoto. 1992 "Role of a two-chain IL-6 receptor system in immune and hematopoietic cell regulation." *Crit Rev.Immunol.* 11(5): 265-280.

118. Tourtellotte, W. W. et al. 1980 "Multiple sclerosis: measurement and validation of central nervous system IgG synthesis rate." *Neurology* 30(3): 240-244.

119. Traggiai, E. et al. 2004 "An efficient method to make human monoclonal antibodies from memory B cells: potent neutralization of SARS coronavirus." *Nat.Med.* 10(8): 871-875.

120. Trapp, B. D., R. Ransohoff, and R. Rudick. 1999 "Axonal pathology in multiple sclerosis: relationship to neurologic disability." *Curr.Opin.Neurol.* 12(3): 295-302.

121. von Budingen, H. C. et al. 2004 "Frontline: Epitope recognition on the myelin/oligodendrocyte glycoprotein differentially influences disease phenotype and antibody effector functions in autoimmune demyelination." *Eur.J.Immunol.* 34(8): 2072-2083.

122. Walsh, M. J. and W. W. Tourtellotte. 1986 "Temporal invariance and clonal uniformity of brain and cerebrospinal IgG, IgA, and IgM in multiple sclerosis." *J.Exp.Med.* 163(1): 41-53.

123. Wardemann, H. et al. 2003 "Predominant autoantibody production by early human B cell precursors." *Science* 301(5638): 1374-1377.

124. Weitkamp, J. H. et al. 2003 "Generation of recombinant human monoclonal antibodies to rotavirus from single antigen-specific B cells selected with fluorescent virus-like particles." *J.Immunol.Methods* 275(1-2): 223-237.

125. Wilske, B. et al. 1986 "Intrathecal production of specific antibodies against Borrelia burgdorferi in patients with lymphocytic meningoradiculitis (Bannwarth's syndrome)." *J.Infect.Dis.* 153(2): 304-314.

126. Wolswijk, G. 1998 "Chronic stage multiple sclerosis lesions contain a relatively quiescent population of oligodendrocyte precursor cells." *J.Neurosci.* 18(2): 601-609.

127. Wucherpfennig, K. W. and J. L. Strominger. 1995 "Molecular mimicry in T cell-mediated autoimmunity: viral peptides activate human T cell clones specific for myelin basic protein." *Cell* 80(5): 695-705.

128. Wyss, C. 1998 "Flagellins, but not endoflagellar sheath proteins, of Treponema pallidum and of pathogen-related oral spirochetes are glycosylated." *Infect.Immun.* 66(12): 5751-5754.

129. Xiao, B. G., C. Linington, and H. Link. 1991 "Antibodies to myelin-oligodendrocyte glycoprotein in cerebrospinal fluid from patients with multiple sclerosis and controls." *J.Neuroimmunol.* 31(2): 91-96.

130. Yu, X. et al. 2006 "Specificity of recombinant antibodies generated from multiple sclerosis cerebrospinal fluid probed with a random peptide library." *J.Neuroimmunol.* 172(1-2): 121-131.

131. Zhang, Y. et al. 2005 "Axon reactive B cells clonally expanded in the cerebrospinal fluid of patients with multiple sclerosis." *J.Clin.Immunol.* 25(3): 254-264.

7 ABBREVIATIONS

BAFF	B cell activating factor of the tumor necrosis factor family
Bb	*Borrelia burgdorferi*
BSA	Bovine serum albumin
C	Constant
CD	Cluster of differentiation
CDR	Complementarity-determining region
cePC	Clonally expanded plasma cells
CIS	Clinically isolated symptom
CMV	Cytomegalievirus
CNS	Central nervous system
CSF	Cerebrospinal fluid
DNA	Deoxyribonucleic acid
EAE	Experimental autoimmune encephalomyelitis
E.coli	*Escherichia coli*
EBV	Ebstein-Barr virus
ELISA	Enzyme linked immunosorbent assay
FACS	Fluorescence activated cell sorting
H	Heavy
HHV-6	Human herpesvirus-6
HRP	Horse radish peroxidase
HSV	Herpes simplex virus
Ig	Immunoglobulin
IL	Interleukin
L	Light
LFA-1	Lymphocyte function-associated antigen-1
mAb	Monoclonal antibody
MALDI-TOF	Matrix assisted laser desorption/ionisation - Time of flight
MBP	Myelin basic protein
MOG	Myelin oligodendrocyte glycoprotein
MRI	Magnetic resonance imaging
MS	Multiple Sclerosis

NB	Neuroborreliosis
OCB	Oligoclonal bands
Osp	Outer surface protein
PCR	Polymerase chain reaction
RT	Reverse transcription
RR-MS	Relapsing- Remitting Multiple Sclerosis
SA	Streptavidin
SC	Single cell
SP-MS	Secondary progressive Multiple Sclerosis
SSPE	Subacute sclerosing panencephalitis
Tp	*Treponema pallidum*
VZV	Varizella- Zoster-Virus
V	Variable

8 CURRICULUM VITAE

Name: KUENZLE

Vorname: Sandra

Geburtsdatum: 5th September 1979

Heimatort: Zürich (ZH), Schweiz

Ausbildung

1992-1999 Literargymnasium Rämibühl, Zürich
Eid. Maturität, Typus B

1999-2001 Biologiegrundstudium
Universität Zürich

2001-2004 Biologiefachstudium
ETH Zürich
Diplomarbeit am Institute für Zellbiologie, ETH Zürich unter Aufsicht von Prof. Dr. S. Werner. Titel: "*In vitro* und *in vivo* Untersuchungen zur Expression und Funktion von ADAMTS-1 während der Wundheilung".

Juni 2004 -
August 2007 PhD Student am Neuroscience Center Zürich. PhD am Institute für Neuroimmunologie, Universität Zürich, unter Aufsicht von Prof. N. Goebels. Titel: "Monocloning Oligoclonality - Dissecting the Humoral Immune Response in Central Nervous System Inflammations".

Publikationen

Krampert M, **Kuenzle S**, Thai SN, Lee N, Iruela-Arispe ML, Werner S (2005). ADAMTS1 proteinase is up-regulated in wounded skin and regulates migration of fibroblast and endothelial cells. *J Biol. Chem.* 280, 23844-23852

Kuenzle S*, von Büdingen HC*, Meier M, Harrer MD, Urich E, Becher B and Goebels N (2006). *SK and HCVB contributed equally Pathogen specificity and autoimmunity are distinct features of antigen driven immune responses in neuroborreliosis. *Infection and Immunity,* August 2007, in press.

Hans-Christian von Büdingen*, Melanie D. Harrer*, **Sandra Kuenzle***, Mirjam Meier, Tobias A. Rupprecht and Norbert Goebels. *HCVB, MH and SK contributed equally Clonally expanded plasma cells in the CSF of MS patients produce CNS specific immunoglobulins. Submitted

Christoph Esslinger*, **Sandra Kuenzle***, Irene Abela*, Alfred Zippelius, Dirk Jäger, Alexander Knuth, Roger M. Nitsch*, Holger Moch*, and Norbert Goebels* *CE, SK and IA as well as RMN, HM and NG contributed equally Exploiting the tumor-specific immune response of cancer patients for the isolation of tumor antigen specific human monoclonal antibodies. Submitted

Stipendium

PhD Stipendium von der David und Betty Koetser Foundation for Brain Research für 3 Jahre und Verlängerung für 6 Monate

Konferenzen und Präsentationen

<u>Präsentationen:</u>

2nd annual Young MS Researcher meeting, 2007, Grindelwald: Monocloning oligoclonality: Solving the secrets of oligoclonal bands.

6th Clinical day of research 2007, Zürich: Pathogen specificity and autoimmunity are distinct features of antigen driven immune responses in neuroborreliosis.

Annual Meeting Swiss Society For Allergology And Immunology 2006, Zürich: Monocloning Oligoclonality – or unravelling the secret of oligoclonal bands.

NCCR meeting 2006, Karthause Ittingen: Data blitz: Monocloning Oligoclonality – or unravelling the secret of oligoclonal bands.

Poster Präsentationen:

Charles Rodolphe Brupbacher Symposium 2007, Zürich: Exploiting the human immune response for the generation of tumor antigen specific human recombinant monoclonal antibodies.

NCCR/SSN (Swiss Society for Neuroscience) meeting 2007, Bern: Pathogen specificity and autoimmunity are distinct features of antigen driven immune responses in neuroborreliosis.

6th Clinical day of research 2007, Zürich: Pathogen specificity and autoimmunity are distinct features of antigen driven immune responses in neuroborreliosis.

Annual Meeting Swiss Society For Allergology And Immunology 2006, Zürich: Monocloning Oligoclonality – or unravelling the secret of oligoclonal bands.

NCCR meeting 2006, Karthause Ittingen: Data blitz: Monocloning Oligoclonality – or unravelling the secret of oligoclonal bands.

21st Congress of the European Committee for Treatment and Research in Multiple Sclerosis/ 10th Annual Meeting of the American Committee for Treatment and Research in Multiple Sclerosis, 2005, Thessaloniki, Greece: Human recombinant monoclonal antibodies derived from clonally expanded plasma cells in the cerebrospinal fluid of MS patients display CNS autoreactivity.

NCCR meeting 2005, Karthause Ittingen: Identification and characterization of myelin oligodendrocyte glycoprotein specific B cells from peripheral blood of MS patients.

PhD retreat 2005, Valens: Identification and characterization of myelin oligodendrocyte glycoprotein specific B cells from peripheral blood of MS patients.

ZNZ Symposium 2005, Zürich: Monocloning Oligoclonality – or unraveling the secret of oligoclonal bands.

9 ACKNOWLEDGEMENTS

My first thanks go to Norbert Goebels, for allowing me to pursue my thesis in the exciting field of Neuroimmunology. His continuous support and interest was fundamental for the progress of my work. I appreciated the freedom and trust I received from him as it helped me to become self-motivated and independent.
I am also very grateful to Adriano Fontana, Peter Sonderegger and Burkhard Becher for being member of my PhD committee. I appreciate that I was always able to knock on Burkhard's door to discuss current problems. His comments were always very helpful and motivating.
My special thanks go to Christian von Büdingen for his enthusiasm, intelligence, patience and immense encouragement that taken together make him a great mentor. Scientific discussions with him were always a great pleasure and one of the highlights of my PhD. I have to acknowledge his valuable input, which contributed greatly to the progress of my work.
Additionally, I want to thank all the present and past members of the Goebels lab. Monica, thanks for your cordial friendship, your poems and for being my sport buddy.
For the non-scientific side of my thesis I particularly want to thank all my great friends, who always support me emotionally and share the good times with me.
I especially want to thank my parents for their faith and for always being there for me.